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POTENTIAL NON-GROWTH USES OF rhIGF-I

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INTRODUCTION

Recombinant human insulin-like growth factor (rhIGF)-I, singly or in combination with its binding protein (recombinant human IGF binding protein [rhIGFBP]-3), was recently approved by the U.S. Food and Drug Administration for the treatment of severe short stature (height <–3 standard deviations) caused by primary IGF-I deficiency. In addition to its role as the principal mediator of somatic growth in humans (together with growth hormone [GH]), IGF-I exerts multiple metabolic and organ-specific effects. IGF-I acts by binding the type 1 IGF receptor (IGF1R), an $\alpha_2\beta_2$ transmembrane tyrosine kinase receptor, leading to phosphorylation cascades involving the mitogen-activated protein (MAP) kinase pathway and the phosphoinositide 3 (PI3) kinase/Akt pathway and, through the latter, the mammalian target of rapamycin (mTOR) pathway. Growing appreciation for the pleiotropic actions of IGF-I has expanded its potential therapeutic usefulness beyond height enhancement. The ongoing trials to assess the role and efficacy of this agent and the current state of investigation regarding IGF-I in experimental models are shown in the Table. We herein review the highlights of some pertinent trials of the potential non-growth uses of rhIGF-I.

ENHANCING INSULIN SIGNALING

The effects of rhIGF-I and rhIGF-I/rhIGFBP-3 have been studied in patients with type 1 diabetes (T1DM)[1-5] and type 2 diabetes (T2DM).[6-9] The rationale for the use of these agents in diabetes is based on the

From The Editor's Desk

Dear Colleague:

In the first issue of 2007, volume 23 number 1, the lead article by Drs. Kim and Grimberg deals with a clinical experimental area of interest—namely, the non-growth uses of IGF-I currently under investigation. Most of the ongoing research trials in this field are at the pre-clinical stage. These protocols should be of academic interest to the readers of GGH; perhaps in the future, some of them may be of potential clinical application if proven safe and efficacious. I also want to bring to your attention a most interesting ethical dilemma posed by the paper "Growth Attenuation in Developmental Disabilities." This paper has been widely discussed in the media and lay press (ie, "A Convenient Truth" by Peter Singer, *The New York Times*, January 26, 2007) and in several blogs on the Internet. The lucid editorial comments by Dr. Sandberg bring forth the ethical considerations in a succinct manner. Please let me know if you have encountered similar situations and/or treated such patients.

The printed version of the journal includes 7 additional reviews and the online version includes 10 additional reviews of importance in the field. In addition, please note the the book review written by Dr. Robert Blizzard in the e-section of the journal. The book is titled *Size Matters: How Height Affects the Health, Happiness, and Success of Boys – and the Men They Become* by Steven Hall (Houghton Mifflin, 2006). This book should be a great resource for you and your patients with short stature. There are few books like this one, although another that I have enjoyed and have recommended to my patients was written several years ago, *The Height of Your Life* by Ralph Keys (Warner Books, 1982).

Sincerely,
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disruptions of the GH/IGF axis associated with diabetes mellitus (Figure). In T1DM, GH levels are elevated,[10,11] yet IGF-I is low,[12,13] indicating a potentially impaired hepatic response to GH. Portal insulinopenia is thought to contribute to this impairment of GH signaling.[14] Decreased delivery of insulin to the liver also produces an increase in IGFBP-1 synthesis, a phenomenon observed in both T1DM and T2DM.[12,15-17] In turn, elevated IGFBP-1 concentrations decrease the bioavailability of IGF-I, further diminishing IGF-I signaling. The loss of negative feedback by IGF-I on GH secretion results in even greater GH secretion, which itself is known to cause insulin resistance.[18-21] Low IGF-I exacerbates hyperglycemia by increasing hepatic glucose output.[22] Given the evidence that GH therapy can cause or worsen diabetes,[23] trials of rhIGF-I and rhIGF-I/rhIGFBP-3 have been carried out in patients with T1DM and T2DM. The results of the larger randomized clinical trials of rhIGF-I and rhIGF-1/rhIGFBP-3 are discussed here.

Type 1 Diabetes

Acerini et al[2] reported on 53 young adults with T1DM randomized to 24 weeks of placebo, or 20 or 40 µg/kg daily of rhIGF-I administered as a single evening injection in addition to their usual multiple-injection insulin regimen. Patients receiving 40 µg/kg/day had an approximately 0.5% lower HbA1c at the end of the 24-week treatment period compared to placebo. There was no difference in retinopathy, hypoglycemia, or any other adverse event.

Thrailkill et al[3] found a similar degree of benefit in a randomized, placebo-controlled trial of 223 subjects with T1DM aged 11 to 66 years. For 12 weeks, patients received 2 injections a day of placebo, or rhIGF-I at doses of 40/40, 80/40, or 80/60 µg/kg (AM dose/PM dose). All patients continued their usual split/mix insulin therapy. At the end of 12 weeks, the HbA1c was 0.5% lower in groups treated with rhIGF-I compared to placebo treatment. Treated groups also experienced a reduction of their daily insulin requirements. The number of hypoglycemic events per person per day increased with increasing dose although the differences were not statistically significant (0.14 and 0.23 episodes per subject per day in the placebo and highest dose groups, respectively). These episodes were defined as a blood glucose ≤60mg/dL, or symptoms of hypoglycemia without a blood glucose measurement. Treated groups had more frequent edema, peripheral edema, jaw pain, headache, and arthralgia, which occurred in a dose-related fashion. Although these were considered minor side effects, they were cited as the cause for the higher drop-out rates in the highest dose groups (21% and 29% in the 80/40 and 80/60 groups, respectively, compared to 15% in the placebo group). Also of concern was that 16 of 199 subjects studied had worsening of diabetic retinopathy, and 13 of these 16 were in the 2 highest dose rhIGF-I groups. Furthermore, 17 patients developed new optic disk swelling, an appearance which can be caused by diabetes or by pseudotumor cerebri.

A 2001 subgroup analysis of the 1999 Thrailkill et al study[24] focused on the younger patients (age 11-21 years) and found a similar degree of HbA1c lowering (about –0.7%, $P<0.05$). Again, insulin requirements were reduced in the rhIGF-I treated groups. Worsening of diabetic retinopathy was also observed in this subgroup. Overall, the reports on the 2 largest controlled trials[2,3] of rhIGF-I suggest improved glycemic control albeit—particularly with higher doses—a high frequency of worsening diabetic retinopathy and other adverse effects often leading to discontinuation of treatment.

The effects of rhIGF-I/rhIGFBP-3 have also been studied in T1DM.[4,5] In April 2000, Clemmons et al[4] published results of their randomized cross-over study of 12 adults with T1DM randomized to 2 weeks of placebo or rhIGF-I/rhIGFBP-3 (2mg/kg/day, composed of an equimolar concentration of IGF-I and IGFBP-3, equaling a ratio of 1:4 by weight) delivered by continuous subcutaneous infusion. All subjects continued their home insulin treatment and measured 4 daily blood glucoses. After a 2-week wash-out period, the patients received the opposite therapy. Investigators and subjects were masked to assignment. At the end of the trial, insulin dose decreased 49% in the treatment group compared to placebo, and mean glucose decreased 23%. The HbA1c did not change during the short trial. There was no difference in hypoglycemic events. Edema, arthralgias, and jaw pain did not occur. Retinal exams were not conducted. The authors concluded that rhIGF-I/rhIGFBP-3 may provide improvements in glycemic control without the adverse

Table. Potential Non-growth Uses of rhIGF-I and Their Level of Development

Potential indication	Preclinical data	Non-randomized trials	Randomized controlled trials
Enhancing insulin signaling			
Type 1 Diabetes mellitus	✓		✓
Type 2 Diabetes mellitus	✓		✓
Type A Insulin Resistance	✓	✓	
Rabson Mendenhall Syndrome	✓	✓	
Lipodystrophy	✓	✓	
Diseases of the Central Nervous System			
Dementia	✓		
Hearing loss	✓		
Spinal cord injury	✓		
Cardiovascular disease	✓		
Osteoporosis	✓		✓

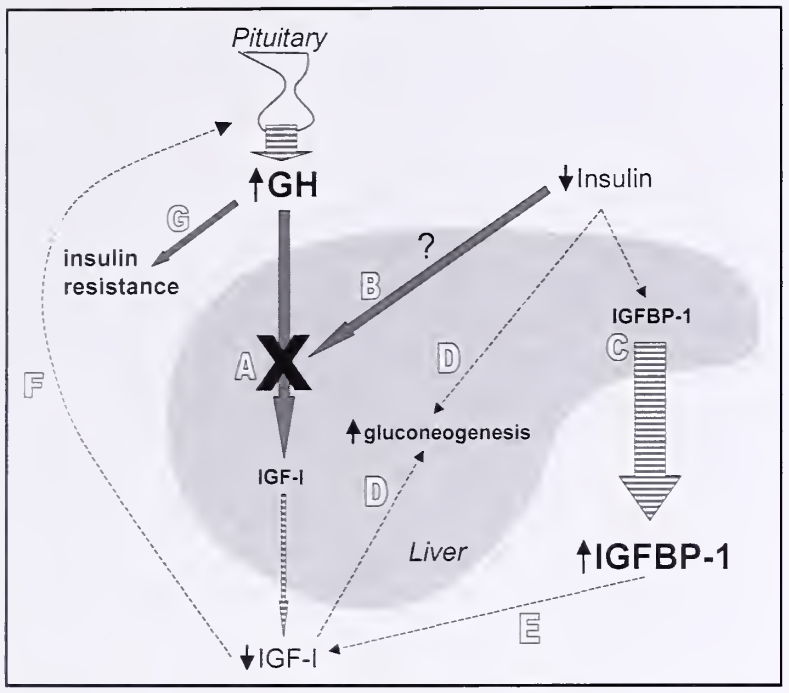

Figure. Disrupted GH/IGF-I signaling in diabetes exacerbates hyperglycemia. Stimulatory pathways are indicated by thick arrows, and inhibitory paths by dashed lines. Striped arrows indicate secretion. **A**. In diabetes, IGF-I is low relative to the GH hypersecretion, ie, a state of GH resistance (indicated by X). **B**. GH resistance may be related to deficient insulin delivery to the liver. **C**. Insulinopenia directly causes increased IGFBP-1 production. **D**. Low IGF-I levels have a permissive effect on hepatic gluconeogenesis, as does insulinopenia itself. **E**. Elevated IGFBP-1 levels decrease the bioavailability and hence activity of IGF-I. **F**. Reduced IGF-I synthesis and bioavailability produce less negative feedback inhibition on GH secretion from the pituitary. **G**. Resultant elevation of GH itself promotes insulin resistance.

effects associated with rhIGF-I alone. However, long-term studies will need to be performed in a larger number of patients to carefully document retinal changes and other side effects, in addition to measures of efficacy.

Type 2 Diabetes and Insulin Resistance

In 2005, Clemmons et al[9] reported on the short-term use of rhIGF-I/rhIGFBP-3 in patients with T2DM. They enrolled 58 adult patients with long-standing (mean 17.1 years) T2DM treated with insulin alone (44 patients) or insulin plus oral hypoglycemic agents (14 patients). The mean baseline HbA1c was 8.2%, fasting glucose was 211 mg/dL, and body mass index was 32 kg/m^2. Subjects were randomized to one of 4 treatment groups: (1) continuous infusion of rhIGF-I/rhIGFBP-3 (2 mg/kg/d); (2) 6h infusion between 2000 and 0200h of 2 mg/kg/d; (3) twice daily subcutaneous injection of 1 mg/kg; or (4) a single evening injection of 1 mg/kg. Patients were hospitalized for the entire 2-week intervention, and all continued their usual home regimen of injected insulin and any oral agents. If needed, insulin was adjusted to "maintain glycemic control." There was no placebo group, and assignment was not masked. Diets were standardized for protein and caloric content, and physical activity was limited to less than 1 mile per day of walking. The mean insulin dose for days 10-14 was reduced in all groups by 54% to 82%. The decrease was significant compared to baseline for all arms, but did not differ from each other, so no difference by delivery method or dose was observed. The mean fasting glucose for days 2-14 decreased by 32% to 37% in all groups. Side effects were frequent, including headache in 19%, hypoglycemia in 10%, back pain in 15%, nausea in 12%, and jaw pain in 4%. Bell's palsy occurred in 1 patient. Six patients dropped out. No retinal exams were conducted. The authors concluded that combination therapy with rhIGF-I/rhIGFBP-3 improves glycemic control in T2DM. The brevity of the study may have reduced the apparent frequency of adverse side effects. However, the major concerns regarding this study are the absence of a placebo group and lack of masking. It is possible, and perhaps likely, that the stringent in-patient protocol consisting of frequent blood glucose monitoring, ensuring compliance with medications, and strict control of diet, by itself would lead to reductions of fasting glucose and insulin dosage in any patient with suboptimal diabetes control.

Other Insulin-Resistant States

Recombinant IGF-I has been studied in metabolic diseases characterized by markedly impaired insulin signaling, including type A insulin resistance,[25-27] Rabson Mendenhall syndrome,[28] and congenital lipodystrophy.[27] These reports uniformly show improvements in parameters of glucose homeostasis, although the numbers of patients studied have been small. Recombinant IGF-I is a logical candidate therapy to enhance glucose homeostasis in these conditions because it acts primarily through the type 1 IGF receptor. Thus, it may provide an "alternate pathway," circumventing a defective or impaired insulin receptor and improving glucose homeostasis through the mechanisms described previously.

Lipodystrophy associated with human immunodeficiency virus (HIV) anti-retrovirals is characterized by insulin resistance, abnormal fat distribution (central fat accumulation and peripheral lipoatrophy), and dyslipidemia. This syndrome is associated with impaired GH and IGF-I secretion.[29,30] Clinical trials have shown that rhGH can induce hyperglycemia while reducing visceral and subcutaneous fat,[31-33] although low doses of rhGH may be a less diabetogenic option.[34] In contrast, rhIGF-I could enhance glucose homeostasis while also improving fat distribution, given evidence that IGF-I may be adipogenic.[35,36] The latter effect could benefit patients with the most severe peripheral lipoatrophy. Currently, the use of rhIGF-I or rhIGF-I/rhIGFBP-3 in the setting of HIV-associated lipodystrophy awaits further study.

THE CENTRAL NERVOUS SYSTEM

IGF-I is essential for normal central nervous system development[37] and function throughout the lifespan, including neuronal plasticity and neuroprotection against potentially pathological disturbances.[38] IGF-I may serve as a regenerative agent in the central nervous system due to its mitogenic and anti-apoptotic actions, whi

stimulate progenitor cell proliferation and the formation and survival of new neurons, oligodendrocytes, and blood vessels.[39] Because IGF-I and insulin are both actively transported from the circulation across the blood-brain barrier,[40] intracranial administration of rhIGF-I, as performed in some experiments, may not be required. Clinical efficiacy of rhIGF-I treatment, systemic or otherwise, remains to be established.

Dementia

Alzheimer disease (AD), the most common form of age-related dementia, is characterized by: (1) extensive brain atrophy from neuronal loss; (2) accumulation of neuritic plaques (deposits of amyloid beta protein); (3) neurofibrillary tangles (aggregates of hyperphosphorylated tau, which misfolds and dissociates from the microtubules); and (4) neuroinflammation surrounding the plaques and tangles. IGF-I has been implicated in affecting all 4 components.[41] Analysis of frontal lobe tissue from brains of AD patients and age-matched controls found that as clinical severity of AD increased, there was decreased expression of insulin, IGF-I, IGF-II, their receptors, tau and Hu D (a neuronal RNA-binding protein that inhibits the decay of labile mRNAs that contain AU-rich elements and affects their nuclear export and translation), but increased expression of disease-related amyloid beta protein precursor and glial fibrillary acidic protein. Choline acetyltransferase expression in insulin receptor and IGF1R positive neurons was also reduced in AD, and increased with insulin or IGF-I stimulation.[42]

The neurodegenerative changes of AD were replicated in vivo by intracerebral streptozotocin (ic-STZ) injection in rats. Without altering peripheral glucose, insulin, or pancreatic status, ic-STZ chemically depleted insulin and IGF signaling and induced oxidative injury within the brain. Brains of rats treated with ic-STZ had reduced size, immunohistochemical changes of neurodegeneration (cell loss, gliosis, and increases in p53, active glycogen synthase kinase [GSK]-3β, phosphorylated tau, and amyloid beta), and changes in gene expression profiles consistent with disease activity.[43] The neurodegenerative changes of AD were also replicated in vivo by blocking IGF1R in the choroid plexus. These rats developed cognitive disturbances, gliosis, synaptic protein loss, brain amyloidosis, and deposits of hyperphosphorylated tau. Restoring IGF1R function mostly corrected these disturbances, and blocking IGF1R exacerbated AD-related pathology in older, already affected mutant mice.[44]

Mechanistically, megalin/low-density lipoprotein receptor-related protein-2 (LRP2) is a multicargo transporter expressed by the choroid plexus that is involved in IGF-I transport into the brain and mediates IGF-induced clearance of brain amyloid beta. Levels of choroid plexus megalin/LRP2 in normal animals were reduced by aging and increased by physical exercise.[45] Premature cerebral accumulation of amyloid beta was found in mice with hepatic-specific *IGF1* gene deletion, while subcutaneous chronic infusion of IGF-I to aged rats promoted amyloid beta levels to decrease in the brain parenchyma and increase in the cerebrospinal fluid.[46] IGF-I treatment was also able to reduce amyloid beta levels in the brains of mice over-expressing mutant amyloid.[46] In addition to megalin/LRP2, albumin and transthyretin have been implicated in IGF-mediated clearance of brain amyloid beta through the choroid plexus, and this function was inhibited by intracarotid injection of tumor necrosis factor alpha (TNF-α), a pro-inflammatory cytokine involved in neurodegeneration.[46] Regarding neurofibrillary tangles, IGF-I can reduce tau phosphorylation directly or indirectly through its effects on amyloid beta.[41] IGF-I also promotes neurogenesis and neuronal survival in adult brains.[47]

The administration of IGF-I showed promising results in several animal models of dementia. Over-expression of mutant amyloid beta precursor protein (APP) and presenilin (PS)2 in transgenic mice results in AD-like cognitive deficits and severe brain amyloidosis. Systemic, slow-release IGF-I treatment of one-year-old, neurologically affected APP/PS2 mice improved cognitive performance, increased levels of synaptic proteins, and decreased brain amyloid beta load and its associated gliosis.[44] A 14-day infusion of amyloid beta 25-35 into the cerebroventricles of rats decreased somatostatinergic signaling in the temporal cortex (a system commonly affected in AD), decreased levels of phosphorylated Akt and increased cell death; these changes were prevented by simultaneous subcutaneous infusion of IGF-I.[48]

Brain atrophy and dementia have also been associated with the catabolic state of diabetes. Like AD, it has been associated with lower IGF-I levels. Subcutaneous infusion of IGF-I in rats with 12 weeks of uncontrolled STZ-induced diabetes partially prevented the loss of brain protein content (by 27.3%), despite ongoing hyperglycemia.[49] Subcutaneous IGF-I infusion in diabetic rats also improved learning/memory performance without ameliorating the hyperglycemia, catabolism or reductions in both total brain and hippocampal weights induced by subcutaneous STZ injection.[50]

Hearing Loss

In addition to short stature and neurodevelopmental delays, sensorineural deafness has been reported in individuals with *IGF1* gene mutation[51] or homozygous partial deletion.[52] Homozygous *IGF1*$^{-/-}$ mice had all-frequency bilateral sensorineural hearing loss and delayed response to acoustic stimuli that increased along the auditory pathway, thereby indicating involvement of both cochlear and central nervous system function.[53] A biodegradable hydrogel, immersed with rhIGF-I or saline for control, was applied to the round window membranes

of Sprague-Dawley rats, 3 days before 2 hours of exposure to 120 dB of white noise. The local rhIGF-I administration significantly blunted the noise-induced elevation of threshold on auditory brain stem response testing (a marker of cochlear function) one week and one month later, and significantly prevented loss of outer hair cells in the temporal bones.[54] These preliminary results suggest IGF-I may be protective for the hearing apparatus against noise-induced damage. These studies also raise speculation about the possible contribution of the age-related decline in circulating IGF-I levels to presbycusis.

Spinal Cord Injury

Moderate voluntary physical exercise can be induced in rats through enriched housing, wherein water and food are placed on opposite sides of the cage and additional attributes, such as running wheels, climbing frames and tubes, are provided.[55] Enriched housing also stimulates the recovery of locomotion after spinal cord injury in rats by inducing voluntary locomotor training. Locomotor training in turn provides locomotor-specific sensory feedback to the central pattern generators that stimulate remodeling of the central nervous system pathways. Subcutaneous rhIGF-I treatment improved locomotor recovery after spinal cord injury in rats, compared to control rats receiving saline infusion, while neutralization of circulating IGF-I with a chronic infusion of anti-IGF-I serum inhibited the benefits of enriched environment on functional recovery.[56]

CARDIOVASCULAR DISEASE

The importance of the GH/IGF axis for cardiovascular health was first indicated by the increased cardiovascular mortality of individuals with GH deficiency.[57] In vitro mechanistic studies further suggested potential benefits from rhIGF-I administration in myocardial disease. IGF-I treatment of cardiac myocytes in culture was shown to attenuate apoptosis induced by hyperosmotic stress; the protective effects of IGF-I required the CREB transcription factor, which was itself activated through the MAP kinase, PI3 kinase, calcium/calmodulin kinase, and calcineurin systems.[58] IGF-I was also shown to protect adult rat ventricular myocytes from high glucose-induced contractile impairments; this required the PI3 kinase/Akt/mTOR pathways but not the calcineurin system.[59]

While GH/IGF-I deficiency portends a worsened cardiovascular prognosis, so too does GH/IGF-I excess; ventricular hypertrophy is a recognized complication of acromegaly[60] and pituitary gigantism.[61] Thus, the conceptual approach of rhIGF-I treatment for cardiovascular remodeling and regeneration, such as the use of rhIGF-I as a myocyte survival and mitogenic factor after myocardial infarction, may be limited by dose-response or situation-dependent toxicities. For example, hypertrophy can be physiologic and adaptive (response to aerobic exercise) or pathologic (response to pressure or volume overload). An intriguing checkpoint has recently been identified. In cultured rat neonatal cardiomyocytes exposed to cyclic mechanical stretch, IGF-I was shown to mediate the induction of myostatin through the stress-activated p38 MAP kinase.[62] A member of the transforming growth factor (TGF)-β superfamily, myostatin is a negative regulator of myocyte growth. Thus, IGF-I and myostatin form a negative feedback loop to regulate cardiac tissue size.[63] Understanding how this balance is achieved and how it can be manipulated will be important for developing effective and safe therapeutic strategies.

Studies of GH, IGF-I, or GH-releasing peptides to treat cardiomyopathies are summarized elsewhere.[64] While there have been clinical trials of rhGH, hexarelin, and ghrelin, studies of rhIGF-I, either singly or in combination with rhGH, are limited to rats following left coronary artery ligation, a common experimental model of post-myocardial infarction heart failure. These studies have shown that in rats with prior coronary artery ligation, rhIGF-I can increase ventricular mass and cardiac output, and lower systemic vascular resistance.[65-68] Results in humans, however, have not been published.

Another role of IGF-I may be to stimulate cardiac tissue regeneration in the setting of stem cell transplantation. Heterologous bone marrow cells were transplanted into the myocardial scars of Lewis rats 3 weeks following experimental ischemia; co-transfection of the donor cells with the genes for IGF-I and vascular endothelial growth factor (VEGF) resulted in better transplanted cell survival, lower apoptosis, and greater left ventricular ejection fraction than cells transfected with either gene singly or control transplantation with cell-free medium.[69] Cardiomyocytes derived from human embryonic stem cells proliferate in vitro, slowing gradually with increasing differentiation. In vitro proliferation of human stem cell-derived cardiomyocytes was inhibited by IGF1R-neutralizing antibodies and dose-dependently enhanced by IGF-I or IGF-II treatment.[70]

OSTEOPOROSIS

IGF-I plays a role in promoting bone anabolism.[71] A pilot, randomized, double-blind, placebo-controlled trial of short-term, continuous subcutaneous infusion of rhIGF-I/rhIGFBP-3 was performed in older women (65-90 years of age) recovering from recent hip fracture. The infusion, administered via portable mini-pump, was initiated within 72 hours of the fracture event and continued for 8 weeks after hip fracture surgery.[72] Thirty patients were randomized 1:1:1 to higher dose (1 mg/kg/day), lower dose (0.5 mg/kg/day) or placebo infusions, and evaluated 6 months post-operatively (ie, 4 months after discontinuation of the infusion). Following the immediate post-operative loss of hip bone density (measured in the contralateral side), the high dose group regained femoral bone density while the placebo group remained

with a deficit at 6 months' follow-up; changes from baseline were –2.6% (P = 0.53) in the former and –6.1% (P<0.05) in the latter. The high dose group also benefited from an 11.4% increase in grip strength (P<0.05), while the placebo group lost 11.6% (P = 0.16), which further contributed to their functional recovery. The rhIGF-I/rhIGFBP-3 was tolerated well at both doses.[72]

Studies of rhGH and/or rhIGF-I, alone or in combination with anti-resorptive drugs, in the treatment of osteoporosis were recently summarized.[73] Controlled trials establishing an effect on fracture incidence are still needed. Also, with multiple options available for the treatment of osteoporosis, cost-effectiveness analyses of using rhIGF-I will need to be considered. For example, a similar short-term, randomized, double-blind, placebo-controlled study was done to address the protein malnutrition that is frequently found in osteoporotic, elderly individuals.[74] Subjects with recent osteoporotic hip fracture (n =82, mean age 80.7 ± 7.4 years) received 550 mg/day calcium supplementation and a single 200000 IU dose of vitamin D at baseline, and were then randomized to receive 20 gm/day protein supplementation or an isocaloric placebo for 6 months. The protein-supplemented group had significant increases in their serum IGF-I levels (85.6 ± 14.8%, vs 34.1 ± 7.2% among controls; P<0.005) at 6 months, less proximal femoral bone mineral loss at 12 months (–2.29 ± 0.75% vs –4.71 ± 0.77% among controls; P<0.05), and shorter median stay in rehabilitation wards (33 vs 54 days; P<0.05). Thus, there may be less expensive interventions than rhIGF-I or rhIGF-I/rhIGFBP-3 to achieve similar therapeutic goals.

NEGATIVE CLINICAL TRIALS OF rhIGF-I

Despite promising animal data or theoretical appeal of rhIGF-I for certain conditions, the results of some clinical trials have shown no benefit. For example, rhIGF-I was shown to enhance recovery in a rat model of acute renal failure.[75] However, a clinical trial of rhIGF-I showed no benefit in human subjects with delayed graft function following cadaveric renal transplantation.[76] Another trial of rhIGF-I was conducted based on the association of aging with lower IGF-I levels. In this study, 16 healthy, non-obese, post-menopausal women (mean age 71 years) were randomized to 1 year of rhIGF-I at a dose of 15 µg/kg given twice daily. The mean circulating IGF-I increased from 66 ng/ml at baseline to 298 ng/ml at 12 months. However, at the end of the study there were no significant differences in bone mineral density, muscle mass, or cognitive function.[77] These studies highlight the critical importance of rigorous testing of all potential uses of rhIGF-I and rhIGF-I/rhIGFBP-3 by randomized, controlled trials.

POTENTIAL SAFETY ISSUES

The adverse effect most commonly encountered in trials of rhIGF-I is hypoglycemia. This can be mitigated by taking the medicine with food. The greatest theoretical risk of rhIGF-I treatment is that of cancer. Not only have higher circulating IGF-I levels been associated with increased risk of multiple cancers, but mechanistically, IGF signaling can contribute to all stages of the neoplastic process.[78] Current evidence supports a permissive—not causal—role for either GH or IGF-I in cancer development.[79] This has been borne out in formal carcinogenicity studies; over-expression of IGF-I in animals or the administration of rhIGF-I increased food intake, body size, and the growth rate of existing tumors, but did not increase tumor incidence.[80] One must keep this effect in mind when considering IGF-I as potential treatment for mature individuals at ages in which cancer incidence is highest to begin with. Careful screening and patient selection, as well as a thorough risk-benefit analysis for each patient, are warranted.

SUMMARY

Recombinant human IGF-I therapy, singly or in combination with rhIGFBP-3, remains experimental for non-growth indications (Table). In diabetes, the best studied of the non-growth clinical indications, rhIGF-I clearly showed enhanced glycemic control in T1DM, but the benefit is offset by a worsening of diabetic retinopathy and a high frequency of other adverse effects including jaw pain, arthralgias, and edema. This is especially concerning at higher doses. In limited studies, low dose rhIGF-I therapy (40µg/kg daily or twice daily) appears to improve glycemic control to a similar extent as higher doses but with fewer side effects. Given the risks of high dose rhIGF-I therapy, robustly designed long-term clinical trials of low-dose rhIGF-I therapy would be required to ensure its efficacy and safety. Combination rhIGF-I/rhIGFBP-3 therapy appears to improve glycemic control in patients with T1DM, although its efficacy in T2DM remains somewhat undefined. The safety of rhIGF-I/rhIGFBP-3 when used for more than 2 weeks is not clear and requires further study. Longer-term, placebo-controlled, double-masked efficacy studies need to be conducted before rhIGF-I is considered for either T1DM or T2DM. Any such studies should include careful interim assessments of microvascular complications and other adverse effects. Additionally, these agents are particularly appealing for insulin-resistant conditions such as lipodystrophy and when there is defective insulin receptor signaling. IGF-I may play a role in other conditions where undesired lean mass catabolism occurs with insulin resistance, as in low-calorie diets for obesity, and where there is severe systemic stress, such as renal disease, burns, cachexia, and other critical illnesses. However, there are no published studies available to date.

Potential use of rhIGF-I for central nervous system disease is promising, especially in light of the paucity of current treatment options. The fact that IGF-I stimulates clearance of brain amyloid beta, thereby correcting what is believed to be the primary pathogenic event, makes

it an especially appealing candidate treatment for AD. However, current evidence is still limited to preliminary preclinical data. While there are a few favorable trials of rhIGF-I in animal models of ischemic myocardial disease, much work needs to be done to determine the patient characteristics and treatment parameters that optimize efficacy and safety. Administration of rhIGF-I improves bone mineral density in some studies, but impact on fracture incidence and cost-effectiveness still needs to be resolved. Other conditions for which rhIGF-I has been found to be unhelpful are in the recovery of renal function after ischemia and as an anti-aging medicine. Potential benefits of IGF-I therapy must always be weighed against the potential risks of raising IGF-I levels. Thus, clinical use of rhIGF-I, singly or in combination with rhIGFBP-3, for non-growth indications should be limited to well-designed clinical studies until adequate evidence supporting efficacy and safety can be collected.

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References

1. Cheetham TD, Dunger DB. Diabet Med. 1995;12:885-92.
2. Acerini CL, Patton CM, Savage MO, et al. Lancet. 1997.350:1199-1204.
3. Thrailkill KM, Quattrin T, Baker L, et al. Diabetes Care. 1999;22:585-92.
4. Clemmons DR, Moses AC, McKay MJ, et al. J Clin Endocrinol Metab. 2000;85:1518-24.
5. Saukkonen T, Amin R, Williams RM, et al. J Clin Endocrinol Metab. 2004;89:4634-41.
6. Zenobi PD, Jaeqqi-Groisman SE, Riesen WF, et al. J Clin Invest. 1992;90:2234-41.
7. Moses AC, Young SC, Morrow LA, et al. Diabetes. 1996;45:91-100.
8. Cusi K, DeFronzo R. J Clin Endocrinol Metab. 2000;85:3077-84.
9. Clemmons DR, Moses AC, Sommer A, et al. Growth Hormone IGF Res. 2005;15:265-74.
10. Zadik Z, Kayne R, Kappy M, et al. Diabetes. 1980;29:655-58.
11. Edge JA, Dunger DB, Matthews DR, et al. J Clin Endocrinol Metab. 1990;71:1356-62.
12. Bereket A, Lang CH, Blethen SL, et al. J Clin Endocrinol Metab. 1995;80:1312-7.
13. Radetti G, Paganini G, Antoniazzi F, et al. Horm Res. 1997;47:110-5.
14. Thrailkill K, Quattrin T, Baker L, et al. J Clin Endocrinol Metab. 1997;82:1181-7.
15. Frystyk J, Skjaerbaek C, Vestbo E, et al. Diabetes Metab Res Rev. 1999;15:314-22.
16. Bang P, Brismar K, Rosenfeld RG, Hall K. J Clin Endocrinol Metab. 1994;78:960-7.
17. Clauson PG, Brismar K, Hall K, et al. Scand J Clin Lab Invest. 1998;58:353-60.
18. Takeda S, Podskalny JM, Gorden P. Metabolism.1984;33:658-61.
19. Johansson Jo, Fowelin J, Landin K, et al. Metabolism. 1995;44:1126-9.
20. Aman J, Kroon M, Karlsson I, et al. Acta Paediatr. 1996;85:31-7.
21. Smith TR, Elmendorf JS, David TS, et al. Am J Physiol Endocrinol Metab. 1997;272:E1071-9.
22. Simpson HL, Jackson NC, Shojaee-Moradie F, et al. J Clin Endocrinol Metab. 2004;89:425-32.
23. Cutfield WS, Wilton P, Bennmarker H, et al. Lancet. 2000;355:610-13.
24. Quattrin T, Thrailkill K, Baker L, et al. J Pediatr Endocrinol Metab 2001;14 267-77.
25. Schoenle EJ, Zenobi PD, Torresani T, et al. Diabetologia. 1991;34:675-9.
26. Morrow LA, O'Brien MB, Moller DE, et al. J Clin Endocrinol Metab. 1994;79:205-10.
27. Kuzuya H, Matsuura N, Sakamoto M, et al. Diabetes. 1993;42:696-705.
28. Quin JD, Fisher BM, Paterson KR, et al. N Engl J Med. 1990;323:1425-6.
29. Rietschel P, Hadigan C, Corcoran C, et al. J Clin Endocrinol Metab. 2001;86:504-10.
30. Vigano A, Mora S, Brambilla P, et al. AIDS. 2003;17:1435-41.
31. Wanke C, Gerrior J, Kantaros J, et al. AIDS. 1999;13:2099-103.
32. Schwarz JM, Mulligan K, Lee J, et al. J Clin Endocrinol Metab. 2002;87:942.
33. Engelson ES, Glesby MJ, Mendez D, et al. J Acquir Immune Defic Syndr. 2002;30:379-91.
34. Lo JC, Mulligan K, Noor MA, et al. Clin Infect Dis. 2004;39:732-5.
35. Bluher S, Kratzsch J, Kiess W. Best Pract Res Clin Endocrinol Metab. 2005;19:577-87.
36. Laron Z, Ginsberg S, Lilos P, et al. Growth Horm IGF Res. 2006;16:61-4.
37. Popken GJ, Dechert-Zeger M, Ye P, et al. Adv Exp Med Biol. 2005;567:187-220.
38. Torres Aleman I. Adv Exp Med Biol. 2005;567:243-58.
39. Aberg ND, Brywe KG, Isgaard J. ScientificWorldJournal. 2006;6:53-80.
40. Yu Y, Kastin AJ, Pan W. Endocrinology. 2006;147:2611-5.
41. Gasparini L, Xu H. Trends Neurosci. 2003;26:404-6.
42. Rivera EJ, Goldin A, Fulmer N, et al. J Alzheimers Dis. 2005;8:247-68.
43. Lester-Coll N, Rivera EJ, Soscia SJ, et al. J Alzheimers Dis. 2006;9:13-33.
44. Carro E, Trejo JL, Gerber A, et al. Neurobiol Aging. 2005;27:1250-7.
45. Carro E, Spuch C, Trejo JL, et al. J Neurosci. 2005;25:10884-93.
46. Carro E, Trejo JL, Gomez-Isla T, et al. Nat Med. 2002;8:1390-7.
47. Trejo Jl, Carro E, Lopez-Lopez C, et al. Growth Horm IGF Res. 2004;14 Suppl A:S39-43.
48. Aguado-Llera D, Arilla-Ferreiro E, Campos-Barros A, et al. J Neurochem. 2005;92:607-15.
49. Lupien SB, Bluhm EJ, Ishii DN. Neurobiol Dis. 2006;21:487-95.
50. Lupien SB, Bluhm EJ, Ishii DN. J Neurosci Res. 2003;74:512-23.
51. Bonapace G, Concolino D, Formicola S, et al. J Med Genet. 2003;40:913-7.
52. Woods KA, Camado-Hubner C, Barter D, et al. Acta Paediatr Suppl. 1997;423:39-45.
53. Cediel R, Riquelme R, Contreras J, et al. Eur J Neurosci. 2006;23:587-90.
54. Iwai K, Nakagawa T, Endo T, et al. Laryngoscope. 2006;116:529-33.
55. Lankhorst AJ, ter Laak MP, van Laar TJ, et al. J Neurotrauma. 2001;18:203-15.
56. Koopmans GC, Brans M, Gomez-Pinilla F, et al. Eur J Neurosci. 2006;23:1035-46.
57. Colao A, Di Somma C, Savanelli MC, et al. Growth Horm IGF Res. 2006;16 Suppl:41-8.
58. Maldonado C, Cea P, Adasme T,et al. Biochem Biophys Res Commun. 2005;336:1112-8.
59. Li SY, Fang CX, Aberle NS 2nd, et al. J Endocrinol. 2005;186:491-503.
60. Colao A, Pivonello R, Marzullo P, et al. J Endocrinol Invest. 2005;28:65-77.
61. Bondanelli M, Bonadonna S, Ambrosio MR, et al. Metabolism. 2005;54:1174-80.
62. Shyu KG, Ko WH, Yang WS, et al. Cardiovasc Res. 2005;68:405-14.
63. Gaussin V, Depre C. Cardiovasc Res. 2005;68:347-9.
64. Marleau S, Mulumba M, Lamontagne D, et al. Cardiovasc Res. 2006;69:26-35.
65. Duerr RI, Huang S, Mirakliakbar HR, et al. J Clin Invest. 1995;95:619-27.
66. Duerr RI, McKirnan MD, Glim RD, et al. Circulation. 1996;93:2188-96.
67. Tivesten A, Caidhal K, Kujacic V, et al. Growth Horm IGF Res. 2001;11:187-95.
68. Tivesten A, Bollano E, Andersson I, et al. Endocrinology. 2002;143:4235-42.
69. Yau TM, Kim C, Li G, et al. Circulation. 2005;112:I123-8.
70. Mcdevitt TC, Laflamme MA, Murry CE. J Mol Cell Cardiol. 2005;39:865-73.
71. Niu T, Rosen CJ. Gene. 2005;361:38-56.
72. Boonen S, Rosen C, Bouillion R. J Clin Endocrinol Metab. 2002;87:1593-9.
73. Agnusdei D, Gentiella R. J Endocrinol Invest. 2005;28:32-36.
74. Schurch MA, Rizolli R, Slosman D, et al. Ann Intern Med. 1998;128:801-9.
75. Ding H, Kopple JD, Cohen A, et al. J Clin Invest. 1993;91:2281-7.
76. Hladunewich MA, Corrigan G, Derby GC, et al. Kidney Int. 2003; 64:593-602.
77. Friedlander Al, Butterfield CE, Moynihan S, et al. J Clin Endocrinol Metab. 2001;86:1496-503.
78. Grimberg A. Cancer Biol Ther. 2003;2:630-5.
79. Grimberg A. Cancer. Adv Exp Med Biol. 2005;567:305-39.
80. Clark RG. Horm Res. 2004;62 Suppl 1:93-100.

REVIEWS & COMMENTS FROM THE LITERATURE

Growth Hormone Deficiency and Antipituitary Antibodies in Celiac Disease

Iughetti et al studied 130 patients (59 males, age 5.67 ± 3.6 years, height 0.32 ± 1.25 SDS) who had been diagnosed with celiac disease (CD) based on the presence of antigliadin, antiendomysial, and antitransglutaminase antibodies, as well as endoscopic biopsies of the distal duodenal mucosa. These children had a poor clinical response to a gluten-free diet (GFD) and a growth hormone deficiency (GHD); they presented to the pediatric clinic at the University of Modena and Reggio Emilia, Italy between 1999 and 2004. Their growth velocity was determined yearly and serum endomysial antibodies were measured after at least 12 months on a GFD. Those children showing no catch-up growth on a GFD were evaluated to exclude possible GHD. Studies included measurement of basal serum GH, insulin-like growth factor (IGF)-I, IGF binding protein (IGFBP)-3, free T_3, free T_4, TSH, prolactin, cortisol, ACTH, LH, FSH, estradiol or testosterone, and repeat studies for antibodies. In addition, antipituitary and antihypothalamus antibodies were measured. On different days, arginine and L-dopa GH stimulation tests were performed in all 7 of the children identified as having poor catch-up growth. Bone age was determined as well. A diagnosis of GHD was based on short stature, decreased growth velocity, delayed skeletal maturation, and blunted GH response (<10 µg/L) to the 2 pharmacological tests. Antipituitary antibodies were detected by an immunofluorescent method that had been previously described. MRIs were performed in these 7 patients.

Five of the 7 patients showed a blunted GH response to the different stimuli and met the criteria for GHD. Four of the 5 had high titers of antipituitary antibodies, 2 were additionally positive for antihypothalamus antibodies. Antipituitary antibodies were also positive in low titers in 3 out of 25 (12%) children with CD only, and in 2 out of 58 (3.4%) control children. None of the 7 children had any pituitary abnormalities on MRI.

The authors stated that in the past an insufficient GH response to hypoglycemia had been reported in children with CD, which subsequently improved with a GFD. The hypothesis that autoimmunity could involve the pituitary gland was reported about 40 years ago; however, the nature and significance of antipituitary antibodies in GHD patients is still being discussed. The authors stated, however, that high titers of antipituitary antibodies could explain some cases of apparent idiopathic GDH. In patients with multiple autoimmune abnormalities, such as the children with CD, these antibodies may explain their GHD.

Iughetti L, De Bellis A, Predieri B, et al. Growth hormone impaired secretion and antipituitary antibodies in patients with celiac disease and poor catch-up growth after a long gluten-free diet period: a causal association? Eur J Pediatr. 2006;165:897-903.

Editor's Comment: *Whether or not one subscribes to the significance of antipituitary antibodies (and/or antihypothalamic antibodies) and the development of isolated GHD, the finding that 5 of 7 children with CD who failed to have catch-up linear growth after 12 months of a GFD met all criteria for GHD is an important finding. Indeed, it is tempting to ascribe failure of catch-up growth following initiation of a GFD to lack of compliance with the meal plan; but, GHD may be present in those children. It is important for pediatric endocrinologists to perform stimulation tests to identify this deficiency. Indeed, low IGF-I levels would not be a surprising finding in children newly diagnosed with CD, as their nutritional status is often poor. However, the failure of IGF-I levels to rise when antiendomysial antibody levels have fallen (at approximately 12 months after the initiation of a GFD) should raise suspicions as to an additional cause for growth failure. As more and more children are being diagnosed with CD, it becomes even more important for the pediatric endocrinologist to be aware of other endocrinologic abnormalities that might be associated with this disease; any of these may be autoimmune in origin.*

William L. Clarke, MD

Genetics of Height Variation

A large number of human genetic disorders including chromosomal and single gene disorders have short stature as a significant component. Aside from these conditions, genetic factors have long been known to influence height within the normal range (ie, short parents have short children). Linkage studies have pointed to a number of chromosomal regions that contain one or more gene(s) that affect height, but the identities of specific genes and how they influence height have eluded investigators. Liu and colleagues offer evidence that height is affected by the interactions of genes located in 2 different chromosomal regions, a phenomenon referred to as epistasis.

Height information was collected on 3726 Caucasians from 434 pedigrees as part of ongoing studies in the Osteoporosis Research Center of Creighton University to

identify genes that contribute to common human traits. Although most of the kindreds contained less than 10 individuals, many were larger, and 14 families had over 40 members who were studied. The many large families provided a large number of relative pairs for height comparisons, which increased the statistical power of the linkage analysis.

Genotyping was performed with microsatellite markers spaced on average about 9 cM apart. The initial analysis was designed to identify individual chromosomal regions linked to height variability. More specifically multipoint and two-point LOD scores were calculated using Sequential Oligogenic Linkage Analysis Routines (SOLAR). The results reported in early 2006 (Liu et al, Hum Genet 2006) suggested linkage for chromosome regions 9q22 and Xq24 and possible linkage at 6p21 and 2q21.

In the second publication reviewed here (Liu et al, J Clin Invest 2006), the investigators further analyzed their data for 3 loci (9q22, 6p21, 2q21) using statistical tests for pairwise epistatic interactions between the 3 loci under different hypothetical models. This approach allowed them to compare the effect of the individual loci with the additive effects of paired loci and with the interactive or epistatic effects of the paired loci. The epistatic model implies a genetic influence that is greater than simply adding the influences of the paired loci together.

The results revealed the most likely model to explain the results is a 2 locus epistatic model involving chromosome regions 6p21 and 2q21. In other words, the analysis suggests that a functional interaction between genes residing in these 2 regions somehow influences height. After making statistical corrections, the authors suggested that the fraction of height variation due to the interaction between these 2 regions is approximately 20%.

The authors discussed the specific gene loci that map to the 6p21 and 2q21 chromosome regions. For example, 6p21 contains genes for the α–2 chain of type XI collagen, the transcription factor, RUNX2 and the retinoid X receptor-ß, RXRB, all of which have known functions in linear skeletal growth. No obvious growth-related genes have been mapped to region 2q21.

Liu YZ, Xiao P, Guo YF, et al. Genetic linkage of human height is confirmed to 9q22 and Xq24. Hum Genet. 2006;119:295-304.

Liu YZ, Guo YF, Xiao P, et al. Epistasis between loci on chromosomes 2 and 6 influences human height. J Clin Endocrin Metab. 2006;91:3821-5.

Editor's Comment: *This report is very interesting, but not very surprising. It is becoming clear from investigations of the skeletal growth plate that its biology and function are regulated by mechanisms that typically involve molecular interactions of multiple gene products (proteins) often in the form of linear pathways, such as signal transduction pathways or in multicomponent complexes, such as signaling platforms and transcriptional complexes that act like complicated machines. Such pathways and complexes provide means for products of different genes to interact functionally, which is presumably what happens in this case, with proteins whose genes map to chromosomes 2 and 6.*

William A. Horton, MD

Adiponectin Suppresses GH and LH In Vitro

Leptin is the prototypic adipokine, a newly recognized class of hormones originating in adipose tissue. Leptin relays the degree of body fatness back to the hypothalamus as part of the homeostatic mechanisms for regulating body energy balance. In addition to its hypothalamic effects, leptin directly stimulates pituitary secretion of leutenizing hormone (LH) and growth hormone (GH), facilitating reproductive, growth, and anabolic functions during times of nutrient abundance.

The more recently discovered adiponectin is the most abundantly secreted of the known adipokines. Adiponectin expression has also been detected in human and murine skeletal muscle, cardiac myocytes, osteoblastic cells, placenta, and chicken pituitaries. Adipocyte expression of both adiponectin and its receptor (AdipoR) have been shown to be regulated, at least in part, by GH.[1,2] Thus, Rodriguez-Pacheco et al sought to investigate whether adiponectin plays a central role, akin to leptin, in regulating somatotroph and gonadotroph function. Anterior pituitary glands were isolated from male Sprague-Dawley rats, minced, enzymatically dissociated and mechanically dispersed to create in vitro cultures of adenohypophyseal cells. After 3 days in culture, and 2 hours in serum-free medium, the pituitary cells were switched to fresh medium and experimental conditions.

Growth hormone secretion was decreased by 34% to 52% after 4 hours incubation in adiponectin at concentrations of 10^{-9} to 10^{-7} M. After 24 hours exposure, only the highest dose of adiponectin (10^{-7} M) changed GH secretion, and that change was a doubling. Focusing on the short-term (4 hours), adiponectin exposure (10^{-7} M) inhibited the stimulation of GH release by 10^{-8} M ghrelin, but not that of 10^{-8} M GH releasing hormone (GHRH). This adiponectin dose increased pituitary cell expression levels of ghrelin receptor (GHS-R) by 34% and GHRH receptors (GHRH-R) by 448%; in the short-term 10^{-8} M adiponectin also significantly induced GHRH-R expression. In contrast, long-term (24 hour) exposure to adiponectin at concentrations of 10^{-9} to 10^{-7} M did not alter expression levels of either GHS-R or GHRH-R.

Like GH, LH secretion was suppressed by 4-hours' incubation in adiponectin at concentrations of 10^{-9} to

10^{-7} M, and the suppression was gone by 24 hours. Four hours of 10^{-7} M adiponectin caused a 74% reduction in the LH secretion stimulated by 10^{-8} M gonadotropin-releasing hormone (GnRH). At 4 hours GnRH receptor expression was halved by adiponectin at concentrations of 10^{-9} to 10^{-7} M, but only the highest concentration of adiponectin significantly reduced GnRH receptor expression at 24 hours.

Rodriguez-Pacheco et al also examined the pituitary adiponectin system. Expression of adiponectin and its 2 receptors (AdipoR1 and AdipoR2) were demonstrated by RT-PCR in extracts of rat and human pituitaries. Returning to the rat pituitary cell culture model, the authors found that 4 hours of adiponectin exposure at concentrations of 10^{-9} to 10^{-7} M increased its own expression by almost 70% (at the highest dose only), but did not alter the expression levels of either of its 2 receptors. However, after 24 hours' exposure, adiponectin (10^{-8} M only) increased its own expression (by 300%), decreased expression of AdipoR1 (by 10^{-8} M only), and increased expression of AdipoR2 (by 10^{-7} M only).

Rodriguez-Pacheco F, Martinez-Fuentes AJ, Tovar S, et al. Regulation of pituitary cell function by adiponectin. Endocrinology. 2007;148:401-10.

Editor's Comment: *Rodriguez-Pacheco et al showed that short-term adiponectin exposure suppressed both basal and stimulated (by ghrelin [but not GHRH] and GnRH) secretion of GH and LH, respectively, by rat pituitary cells in vitro. They further laid the groundwork for a pituitary adiponectin autocrine/paracrine system in which both adiponectin and its receptors are expressed and further modulated by adiponectin exposure. Thus, adiponectin seems to serve like the classic adipokine leptin, in centrally linking growth, anabolic, and reproductive function to fat cell activity. These relationships warrant in vivo confirmation. From the evidence so far, it seems that neither endocrine leptin nor endocrine adiponectin underlie the old clinical observation that obesity suppresses GH secretion; circulating leptin levels are increased in obesity but leptin stimulates GH release, and although adiponectin suppresses GH secretion, as shown in this paper, circulating adiponectin levels are reduced in obesity.*

Nonetheless, adiponectin attracts tremendous clinical interest. Adiponectin seems to do what clinicians are desperately seeking to accomplish in the obesity epidemic: adiponectin acts as an insulin-sensitizing, anti-atherogenic, anti-inflammatory, anti-angiogenic, and anti-tumoral agent. The sooner we learn about adiponectin physiology, the sooner it can inspire novel therapeutic approaches.[3,4] For example, it turns out that thiazolidinediones up-regulate adiponectin. Adiponectin's reported insulin-sensitizing activities are multiple and peripheral: it enhances hepatic insulin action and decreases endogenous glucose production; it increases glucose uptake by adipocytes and myocytes, and it increases fatty acid oxidation in muscle. If the in vitro findings of this paper are confirmed in vivo, then we can add one more mechanism to the list: adiponectin centrally inhibits secretion of the counter-regulatory GH. Further, adiponectin was shown to decrease body weight in mice by stimulating energy expenditure.[5] Not everything from fat is bad.

Adda Grimberg, MD

References

1. Nilsson L, Binart N, Bohlooly YM, et al. Biochem Biophys Res Commun. 2005;331:1120-6.
2. Fasshauer M, Klein J, Kralisch S, et al. FEBS Lett. 2004;558:27-32.
3. Kadowaki T, Yamauchi T, Kubota N, et al. J Clin Invest 2006;116:1784-92.
4. Matsuzawa Y. FEBS Lett. 2006;580:2917-21.
5. Qi Y, Takahashi N, Hileman SM, et al. Nat Med. 2004;10:524-9.

Vitamin D Receptor in Idiopathic Short Stature

Stature is a highly heritable trait, but beyond those genes known to cause specific disorders in which short stature is a major component, the genetic factors responsible for variation in height are poorly understood. As reported by Dempfle et al, genome-wide linkage scans of adult height have been performed in at least 22 separate samples and the results summarized in 12 publications. Although these studies, most of which have been performed on relatively small samples, yielded divergent results and no chromosomal region was highlighted across all scans, evidence for linkage is convincing for some regions, in particular regions on chromosomes 6, 7, 9, and 12.

Building on these studies, Dempfle et al carried out a genome-wide scan on 92 families, each with 2 affected children with idiopathic short stature (ISS), which they defined as including constitutional delay of growth and puberty, familial short stature, and ISS in its more narrow meaning. For inclusion, each family had one child whose height was below the 5th percentile and a second child with height less than the 15th percentile. Only Caucasian families were included, and all but 2 parents were of German origin.

Linkage analysis using 511 short tandem repeat markers revealed the highest LOD score (3.18 [and only LOD score >3]), which is usually accepted proof of linkage, at chromosome 12q11. This is the region to which adult height has been linked and which contains the vitamin D receptor (*VDR*) gene that has been previously implicated as a factor in adult height variability. In fact, as noted in a 2005 GGH abstract,[1] a single nucleotide polymorphism (SNP) at the *VDR* locus has been associated with variation in adult stature. The same association was found in ISS in this investigation.

The *VDR* polymorphism involves the substitution of a G base for an A base at a particular nucleotide; it is called the G allele. The G allele was detected more often

than the A allele in children and adolescents with ISS. The substitution maps to the *VDR* start codon where it abolishes the first translation initiation site, resulting in a peptide lacking 3 amino acids, which increases the transcriptional activity of the gene. The more active allele was over-transmitted to affected children in the sample giving estimates of relative risks for ISS of 1.33 and 1.9, respectively, for heterozygotes and homozygotes for the allele. The authors suggested that on the population level, the G allele might be responsible for 34% of ISS cases.

The genomic scan did not detect evidence of linkage to other sites that have been implicated by other investigators in ISS, including the *SHOX* and *NPR2* loci.

Dempfle A, Wudy SA, Saar K, et al. Evidence for involvement of the vitamin D receptor gene in idiopathic short stature via a genome-wide linkage study and subsequent association studies. Hum Molec Genet. 2006;15:2772-83.

Editor's Comment: *Readers may ask: if linkage to the VDR locus and association with the G allele of VDR has been established for adult height, why repeat the genomic scan in children with ISS? The reason is that the findings in the adult study could be explained by the effects of several genes, each having a small impact on stature or a small number of genes having a larger impact. Finding a similar effect in a small subset of individuals, ie, those with ISS, argues for a larger effect of a smaller number of genes, of which VDR is one. The next step will be to delineate how the more transcriptionally active VDR allele actually affects linear bone growth.*

William A. Horton, MD

Reference

1. Growth Genet Horm. 2005;21:e16. www.gghjournal.com/volume21/3/ab16_pf.cfm

Hyperinsulinemia, Impaired Glucose Tolerance, and T2DM in Cancer Survivors

The occurrence of hyperinsulinism and type 2 diabetes mellitus (T2DM) has been identified in survivors of childhood malignancy, particularly after bone marrow transplantation (BMT). Only small numbers of patients had been studied and evaluated long-term. The recent study of Hoffmeister et al[1] dealing with a population of children followed after hematopoietic cell transplantation, showed a 3-fold increase rate for T1DM and T2DM. The study of Neville et al focused on the predisposing factors and early markers of DM, a critical issue for the development of prevention strategies. This group studied 248 survivors of childhood cancers; half of them were adults at the time of evaluation. The median duration after diagnosis was 12.9 years. They grouped hyperinsulinism (HI), impaired glucose tolerance (IGT), and T2DM together for analysis of potential risk factors. Body mass index (BMI) and abdominal adiposity were potential markers. In this population, which is often growth-retarded, the waist-to-height (W/H) ratio correlated well with the volume of visceral fat as measured by CT scan. A ratio of >0.5 was considered a good predictor of complications of obesity.

The mean BMIs of both prepubertal and pubertal subjects were similar compared with controls, but the mean W/H ratio was higher, with a doubling of the percentage in children with abdominal adiposity. In all groups, there was a tendency for accumulating abdominal fat. In pubertal and adult subjects, abdominal adiposity was predictive for the occurrence of biochemical markers for metabolic abnormalities (insulinemia and lipid profiles). Fasting insulin concentrations were higher in prepubertal and pubertal subjects, compared with their controls. Hyperinsulinism, IGT, or DM were detected in 18% of pubertal and adult subjects. Eleven percent of this group had IGT/DM ($p<0.001$). In the group with BMT, conditioning with total body irradiation (TBI) increased the risk (Table).

This study confirms the risk factors previously identified, with a strong focus on the BMT group. Total body irradiation turns out to be a major risk factor for metabolic abnormalities. Differences with previously reported studies could be accounted for by the prospective approach, tha

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Table. Significant risk factors in cancer survivors for the development of HI, IGT, or DM

	Odds ratio (95% CI)	*P* value
BMT (54) *vs.* all others (158)	6.6 (3.1–13.9)	<0.001
ALL BMT (16) *vs.* no BMT (82)[1]	25.6 (6.6–100)	<0.001
TBI	13.8 (5.7–34.3)	<0.001
Pituitary irradiation (≥ 30 Gy)	4.5 (2.1–10.0)	<0.001
GH deficiency	5.1 (2.3–11.3)	<0.001
Untreated hypogonadism	21.1 (6.4–69.7)	<0.001
Untreated hypothyroidism	19.7 (2.1–181.2)	0.009
Overweight or obese (BMI)	5.3 (2.5–11.4)	<0.001
Abdominal adiposity[2]	14.5 (4.9–42.8)	<0.001
Family history of dyslipidemia	2.1 (1.0–4.2)	0.04
Hypertension	2.6 (1.1–5.8)	0.03
BMT survivors only (54)		
TBI	7.6 (2.2–26.2)	<0.001
Busulphan	0.2 (0.1–0.8)	0.02

[1] Patients with acute lymphoblastic leukemia (ALL) with and without BMT.
[2] Abdominal adiposity defined as a waist-to-height ratio more than 0.5.
(n)=number

Modified from Neville KA, et al. J Clin Endocrinol Metab. 2006;91:4401-7.

broad ranging diagnoses, and the grouping together of the 3 metabolic criteria. Interestingly, hypogonadism also emerged as an independent risk factor, and W/H ratio was a more important marker than BMI. In keeping with these data, it is suggested that the use of conditioning with TBI for BMT deserves reconsideration and underlines the need for regular and long-term clinical and metabolic follow-up.

Neville KA, Cohn RJ, Steinbeck KS, Johnston K, Walker JL. Hyperinsulinemia, impaired glucose tolerance, and diabetes mellitus in survivors of childhood cancer: prevalence and risk factors. J Clin Endocrinol Metab. 2006;91:4401-7.

Editor's Comment: *Diabetes mellitus has not been considered a significant risk in the follow-up of cancer survivors. Initially, treatment with asparaginase suggested a rare immediate risk. Thereafter, the higher frequency of moderate—but significant—overweight observed in patients with leukemia suggested such a risk. In the present prospective study of a large group of etiologies, a new vision is emerging. Of note, some factors did not turn out to be significant: asparaginase-related hyperglycemia, diagnosis, small birth size, abdominal or testicular irradiation. The group at risk had BMT with TBI as conditioning, as opposed to busulfan conditioning, which had no significant effect on the metabolic outcome. The authors suggested that the pancreatic beta cell is an unlikely target, and instead focused on the effect of irradiation on the muscle mass by unknown mechanisms, one possibly being mitochondrial dysfunction. Little is known about the outcome of the irradiated adipose tissue and possible inflammatory processes.*

This study provides some clinical clues such as early correction of hypogonadism and careful follow-up of W/H ratio. In the population at risk because of TBI, appropriate nutritional and lifestyle control may not be sufficient. More long-term studies are needed to help understand the mechanism(s) of these adipose—and possibly muscular—changes to help prevent metabolic syndrome and DM.

Raphaël Rappaport, MD

Reference

1. Hoffmeister PA, Storer BE, Sanders JE. J Pediatr Hematol Oncol. 2004;26:81-90.

Growth Attenuation in Developmental Disabilities

Caring for nonambulatory children with profound developmental and cognitive disabilities becomes more difficult as the child grows. Treatment with high-dose estrogen, can arrest further growth and facilitate the option of continued care in the home. This case report discusses medical and ethical considerations of such an intervention strategy and describes a comprehensive program including reviews by pediatric specialists in endocrinology, neurology, development, surgery, and ethics.

A 6-year, 7-month-old girl was referred to pediatric endocrinology for early pubertal development. She had a 1-year history of pubic hair and a 3-month history of breast budding. Static encephalopathy with marked global developmental deficits was previously diagnosed. Motor and cognitive development never progressed beyond that of an infant; at 6 years of age she could not sit up, ambulate, or use language. She was gastrostomy-tube dependent for nutrition and responded to others by vocalizing and smiling. The consensus of the specialists was that there would be no significant future improvement in cognitive or neurological function.

The onset of puberty roused parental fears that they would not be able to continue to care for their daughter at home, despite their desire to do so, as she continued to grow. A plan to attenuate growth using high-dose estrogen was developed along with pretreatment hysterectomy. An institutional ethics committee met with the family, patient, and patient's physicians and reached consensus that requests for growth attenuation and hysterectomy were ethically appropriate in this case. Plans were instituted to convene an interdisciplinary review panel that included pediatric specialists in

endocrinology, neurology, development, surgery, and ethics. After an uneventful surgery and a little more than a year of daily transdermal estradiol (400 µg), the patient approached the end of her linear growth.

Gunther and Diekema reviewed the history of growth-attenuation therapy, in particular its application to tall adolescent girls. Most reported decreases in adult height between 2 cm and 10 cm, with greatest reductions observed the earlier the treatment was initiated. The authors speculated that "treatment beginning in a 5-year-old boy of average height and weight might result in a reduction in final length of as much as 24 inches (60 cm) and in weight of more than 100 pounds (45 kg)." What of the risks of treatment? Based on experiences in treating girls for constitutional tall stature, known short-term risks of high-dose estrogen treatment include mild nausea, headache, and weight gain. Long-term effects on fertility have recently been raised, although this risk did not apply to the case(s) in question. The effects of high-dose estrogen in young prepubertal children includes gynecomastia in boys, and rapid advancement of secondary sexual characteristics in girls, including uterine bleeding which can be controlled with injections of depot medroxyprogesterone acetate (DMPA) or hysterectomy. Concerns over the risk of thrombosis were not thought to be a reason to withhold this intervention. The authors acknowledged historical controversies associated with hysterectomy but, in profoundly impaired children, careful ethical and legal deliberations are needed.

Ethical factors in the decision to employ high-dose estrogen treatment to attenuate growth in a profoundly impaired individual included past abuses against this population justified by the benefits to society or the caretakers, rather than the individual. The authors discouraged overgeneralizing from past abuses directed toward mildly- to moderately-impaired individuals to the potential benefits of such interventions for those who are nonambulatory, profoundly cognitively and neurologically impaired, and wholly dependent on others for all their needs. Two major considerations in determining whether it is ethical to attenuate growth in this population exist: does growth attenuation offer the patient benefit, and does growth attenuation do any harm to the patient? A thoughtful discussion was presented with the thrust pointing in the direction of benefits to the child and family through the reduced physical burden on aging parents in attending to the changing physical needs of their child in the home. The authors addressed medical and psychosocial harm associated with this treatment, and concluded that patients such as the one being discussed would not be placed unduly at risk.

Gunther DF, Diekema DS. Attenuating growth in children with profound developmental disability: a new approach to an old dilemma. Arch Pediatr Adoles Med. 2006;160:1013-17.

Editor's Comment: *In an editorial accompanying this article, Brosco and Feudtner[1] acknowledged the predicament facing parents of children with profound cognitive and physical disabilities who are considering continuing care in the home. In evaluating the reasoning of Gunther and Diekema's strategy, they posed 4 questions: (1) Will early high-dose estrogen treatment enable such children to remain home under the care of their parents for longer periods of time? Will this improve the quality of their lives? What if the height-attenuated child continues to gain weight; wouldn't this effect partially offset the benefits of shorter stature? These questions, in addition to concern over the association between low-dose estrogen therapy and seizures, lead the commentators to call for a rigorous investigation of assumptions underlying this intervention and examination of unforeseen risks; (2) Is it acceptable to manipulate a person's height? Here, the authors have little trouble dispensing with such concerns; they opined that to not do so implies that a person's value as a human being is dependent upon their physical size; (3) Will this treatment be misused? The history of the eugenic movement in the first half of the 20th century and more recent practices (eg, sterilization of individuals with mental retardation) that have come to be rejected, lead the authors to call for stiff safeguards and protections; and (4) Is the proposed treatment an attempt at a simple technical fix to a far more complex problem, that being the plight of families caring for such children without adequate societal support?*

The authors concluded that attempts to attenuate growth in such cases are ill-advised. Instead, they claim what is needed are more funds for home-based services. They warn clinicians that adopting medical interventions, even in the context of interdisciplinary review with ethical oversight, will ultimately be judged "in the social-political context of both the disability rights movement and the woefully impoverished options for high-quality, long-term residential care of children or adults with profound developmental disabilities."

Personally, I am particularly sympathetic to the fourth point of Brosco and Feudtner. It appears that in our effort to help struggling families, we may narrow our intervention options to those perceived to be in our control, in this case, high-dose estrogen therapy to attenuate growth. Adopting such an approach to achieve the desired outcome of allowing the parents to continue to care for their child at home seems far more attainable than trying to modify the state of home-based services. Besides, strictly speaking, we are not paid to do the latter. However, if there is validity to this line of reasoning, then how do we avoid choosing the expedient and readily available when it clashes with the just course of action?

David E. Sandberg, PhD

Reference

1. Brosco JP, Feudtner C. Arch Pediatr Adoles Med. 2006;160:1077-8.

Sox2/SOX2 Mutation and Abnormalities in Hypothalamo-pituitary-gonadal Axis

Many transcription factors are required for normal development of the adenohypophysis, including *LHX3*, *LHX4*, *HESX1*, *PROP1*, *POU1F1*, *SF1*, *SOX3*, and others. To this growing list may now be added *SOX2* (OMIM 184429, chromosome 3q26.3-q27), another member of the family of transcription factors that contain an SRY-related, 79 amino acid, high-mobility group box (HMG) DNA-binding domain. The 20 members of the SOX gene family encode proteins that are necessary for neuroepithelial cell differentiation. Humans with heterozygous mutations of SOX2 have bilateral anophthalmia/microphthalmia, sensorineural hearing loss, anomalies of the male genital tract, short stature, and developmental delay. To this clinical picture, the present authors have added hypogonadotropic hypogonadism. The investigators examined the *SOX2* genotype in 235 patients (143 male) with congenital hypothalamic-pituitary disorders (97 subjects with congenital hypopituitarism and no midline cranial or eye defects; 126 patients with septo-optic dysplasia [SOD], 12 patients with anophthalmia/microphthalmia). They identified heterozygous loss-of-function mutations in *SOX2* in 8 patients, 6 of whom had bilateral or unilateral anophthalmia/microphthalmia and 2 of whom had SOD (Table). The 6 patients with congenital defects of eye formation had isolated hypogonadotropic hypogonadism, one subject with SOD had deficiencies of growth hormone (GH), TSH, and ACTH, while the second patient with SOD was short but pituitary function had not been evaluated. Depending on the site of the *SOX2* mutation, there was defective nuclear localization of the SOX2 protein, impairment of its binding to DNA, or decrease in its ability to transactivate target genes.

The investigators then evaluated mice in which *Sox2* had been partially inactivated. Homozygous loss of *Sox2* was lethal, while partial heterozygous loss of *Sox2* led to impaired growth and subfertility in males, which was associated with decreased pituitary content of GH and LH (but no ocular abnormalities). The adenohypophyses of these animals were small, the morphology of the somatotrophs and gonadotrophs abnormal, and the number of these cells low compared to wild-type animals. The pituitary contents of TSH and prolactin were variably low in the heterozygous mice, but that of ACTH was normal. In addition, *Sox2* heterozygous male mice were subfertile; many had small testes with abnormal spermatogenesis. The *Sox2* heterozygous female mice had normal fertility. The data indicate that *Sox2* is an important transcription factor for development of the anterior pituitary (and testes/spermatogenesis). The pituitary expression of *Sox2* normally declines as the adenohypophysis develops, but its expression is maintained in the hypothalamus, suggesting that an abnormality of hypothalamic function may also be present in mice with heterozygous mutations in *Sox2*. (With more stringent *Sox2* deficiency, ocular anomalies can be produced experimentally.[1])

Kelberman D, Rizzoti K, Avilion A, et al. Mutations within *Sox2/SOX2* are associated with abnormalities in the hypothalamo-pituitary-gonadal axis in mice and humans. J Clin Invest. 2006.116:2442-55.

Table. Clinical phenotype in patients with *SOX2* mutations

Pt	Mutation	MRI	Ocular phenotype	Other features
1	c.60insG	Hippocampal abnormalities, small corpus callosum, hypothalamic hamartoma, APH, generalized reduction of white matter bulk, absent optic nerves	Bilateral anophthalmia	HH, learning difficulties, spastic diplegia, esophageal atresia
2	c.70del20	Hippocampal abnormalities, abnormal anterior pituitary, absent left optic nerve	Left anophthalmia, right microphthalmia	HH, learning difficulties
3	c.387delC	Hypoplastic corpus callosum, APH, hypothalamic hamartoma, small left optic nerve and chiasm, generalized lack of white matter bulk, hippocampal abnormalities with small and rotated mesial temporal structures	Left microphthalmia, right coloboma	HH, cryptorchidism, micropenis, learning difficulties, mild spastic diplegia
4	Y160X	Partial agenesis of corpus callosum, small anterior pituitary, hippocampal abnormalities, generalized reduction in white matter	Bilateral microphthalmia	HH, cryptorchidism, micropenis, severe learning difficulties, spastic and dystonic quadriparesis
5	Q177X	Not done	Bilateral anophthalmia	HH, cryptorchidism, micropenis, severe learning difficulties, mild facial dysmorphism
6	c.479delA	APH, small hippocampus, thin corpus callosum, cavum septum pellucidum, absence of optic nerves and chiasm	Bilateral anophthalmia	HH, small testes, micropenis, learning difficulties, sensorineural deafness
7	G130A	Absent septum pellucidum, bilateral optic nerve hypoplasia, bilateral schizencephaly, right porencephalic cyst, normal anterior and posterior pituitary	Roving nystagmus with bilateral optic nerve hypoplasia	Short stature with a normal growth velocity; endocrine status not investigated
8	A191T	Absent septum pellucidum, small optic chiasm, absent infundibulum, severe APH, ectopic-undescended posterior pituitary	Roving nystagmus with bilateral optic nerve hypoplasia	GH, TSH, and ACTH deficiency

Editor's Comment: *Loss of SOX3 (OMIM 313430, chromosome Xq26.3) activity in man is associated with X-linked GH deficiency and mental retardation.[2] Reynaud and co-workers[3] reported the distribution of mutations in PROP1, POU1F1, LHX3, LHX4, and HESX1 in a population of 165 unrelated families (195 patients) with deficiencies of multiple anterior pituitary hormones (combined pituitary hormone deficiency [CPHD]) with or without SOD or pituitary stalk interruption syndrome (PSIS). Overall mutations in one of the 5 transcription factor genes examined were found in 22 of 165 index patients (13.3%). CPHD was familial in 21 families, with mutations identified in 10 of these 21 families (52.4%).Homozygous or double heterozygous mutations in PROP1 were identified in 20 patients, in 8 of whom CPHD was familial. A mutation in POU1F1 or LHX4 was identified in only one patient each, and no mutations in LHX4 or HESX1 were found in this CPHD population. Although mutations of HESX1 have been found in patients with SOD, none were identified in the report of Reynaud and co-workers. It would be of interest to analyze SOX2 in these subjects. Reynaud et al also correlated phenotype with genotype and outlined a schematic algorithm through which gene analysis of patients with CPHD and associated anomalies might be pursued (Figure).*

Allen W. Root, MD

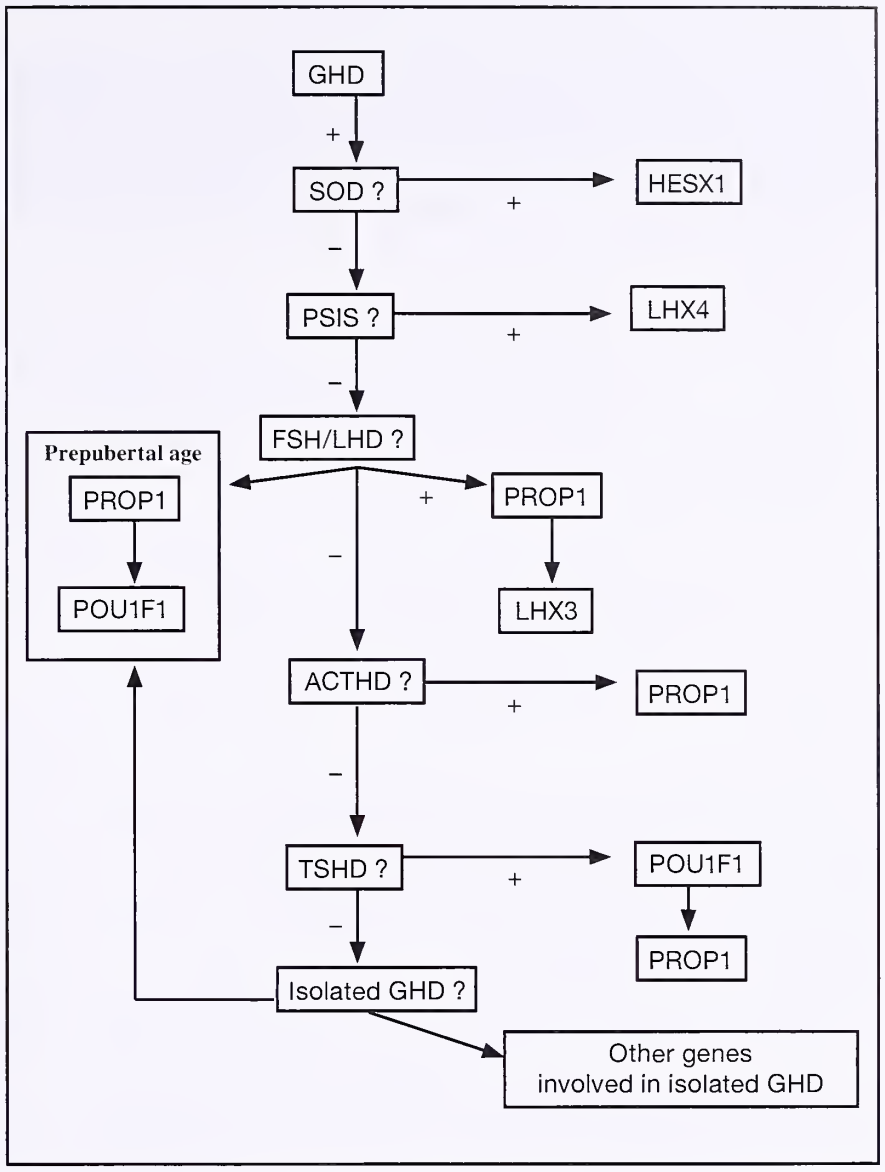

Figure. Algorithm of CPHD genetic screening. FSH/LHD, FSH and LH deficiencies; ACTHD, ACTH deficiency.

References

1. Taranova OV, Magness ST, Fagan BM, et al. Genes Dev. 2006.20:1187-202.
2. Laumonnier F, Ronce N, Hamel BC, et al. Am J Hum Genet. 2002;71:1450-5.
3. Reynaud R, Gueydan M, Saveanu A, et al. J Clin Endocrinol Metab. 2006;91:3329-36.

Clinical Significance of a 6 Hr Exon 3-deletion Polymorphism

Audi and colleagues from the Spanish SGA Study Group reported the relative frequencies of the deleted and full-length exon 3 growth hormone receptor (GHR) polymorphisms in 247 short stature children and adolescents with birth weight small for gestational age (SGA) and 289 normal stature adult control subjects. The homozygous or heterozygous inheritance of the exon 3 deleted isoform has been reported to enhance GH action, although the significance of this genotype on GHR function is unknown. There was a 2-fold increase of the biologically less active homozygous full-length exon 3 isoform genotype in the SGA subjects. In the control population, there was no relationship between the height phenotypes and genotypes of the subjects. Therefore, it is suggested that in short stature SGA subjects, the presence of the full-length isoform may have impeded post-natal catch-up growth.

Carrascosa et al, also from the Spanish SGA Hormone Study Group, reported the results of GH therapy in patients from the same cohort of SGA subjects as described by Audi and colleagues. Previous reports have demonstrated an increased growth response to GH therapy in SGA subjects who have the deleted exon 3 isoform, compared to those with the full-length receptor.[1] In contrast, this paper reported no differences in first- or second-year growth velocity and height gain between the different genotypes of 86 GH-treated SGA subjects. These patients were treated with a GH dose of 66 µg/kg/day, an amount that is at the upper end of the recommended dose and higher than in other reported series. It was suggested that these high GH doses might over-ride a more subtle effect reported with lower GH regimens.

Jorge and colleagues from São Paulo, Brazil performed a retrospective genetic analysis for the retained or deleted exon 3 GHR genotypes in 75 patients with severe isolated or combined GH deficiency. Clinical and laboratory data were similar at baseline in patients with different

genotypes. However, patients on GH therapy who were carrying at least one GHRd3 allele demonstrated a higher first-year height velocity ($P<0.05$), compared to those with the full-length isoform. Final height was also greater in the GHRd3 subjects. No parental height data were given. Jorge et al hypothesized that manipulation of GH dose following genotype characterization might become a reality in the future.

Blum and colleagues from Eli Lilly in Germany studied 107 patients with severe idiopathic GH deficiency. In contrast to the Jorge group, they found no difference in growth responses to GH therapy between the subjects with the d3-GHR allele and those with the full-length receptor.

Audi L, Esteban C, Carrascosa A, et al. Exon 3-deleted/full-length growth hormone receptor polymorphism (*d3/fl*-GHR) genotype frequencies in Spanish short small-for-gestational-age (SGA) children and adolescents (n=247) and in an adult control population (n=289) show increased *fl/fl* in short GSA. J Clin Endocrinol Metab. 2006;91:5038-43.

Carrascosa A, Esteban C, Espadero R, et al. The d3/fl-growth hormone (GH) receptor polymorphism does not influence the effect of GH treatment (66 microg/kg per day) or the spontaneous growth in short non-GH-deficient small-for-gestational-age children: results from a two-year controlled prospective study in 170 Spanish patients. J Clin Endocrinol Metab. 2006;91:2381-6.

Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, Arnhold IJ. Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. J Clin Endocrinol Metab. 2006;91:1076-80.

Blum WF. Machinis K, Shavrikova EP, et al. The growth response to growth hormone (GH) treatment in children with isolated GH deficiency is independent of the presence of the exon 3-minus isoform of the GH receptor. J Clin Endocrinol Metab. 2006;91:4171-4.

Editor's Comment: *Several large studies that look at the possible influence on responses to GH therapy of homozygous or heterozygous inheritance of the deleted exon 3 GHR isoform have now been performed. The results are conflicting in SGA subjects as no difference in growth response was found in the Spanish study, contrasting with the original description of an apparent growth-enhancing effect shown in the French study. However, Binder et al[2] reported significantly increased responses in both SGA and Turner syndrome patients carrying the exon 3-deleted isoform. Now, Jorge and Blum have reported different outcomes and conclusions in patients with GH deficiency.*

With such differing conclusions, it is hard to imagine that an effect of real clinical relevance exists from the inheritance of the deleted isoform. No doubt further studies will be published with probably differing conclusions. At this stage, the prospective genotyping of short patients in order to optimize their responses to GH therapy seems premature.

Martin O. Savage, MD

References

1. Dos Santos C, Essioux L, Teinturier C, et al. Nat Genet. 2004;36:720-4.
2. Binder G, Baur F, Schweizer R, et al. J Clin Endocrinol Metab. 2006;91:659-64.

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GROWTH IN OSTEOGENESIS IMPERFECTA

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INTRODUCTION

Osteogenesis imperfecta (OI), or brittle bone disease, is a rare disorder with congenital bone fragility caused by mutations in the genes that codify for type I pro-collagen production in osteoblasts (*COL1A1* and *COL1A2*), located in chromosomes 7 and 17.[1] Numerous mutations have been described as causing the condition.[2] In the vast majority of cases, OI is inherited in a dominant fashion, or caused by a new mutation. The prevalence of OI is estimated to be 1 in 20 000 to 50 000 infants.[3]

Besides brittle bones, clinical characteristics and severity of OI are widely variable. There may even be a different degree of severity in different members of the same family.[4,5] Clinical features that may be present include bone fragility, joint hyperlaxity, muscle weakness, chronic unremitting bone pain, and skull deformities (eg, posterior flattening) due to bone fragility in infants with severe OI. Fractures may still occur after puberty,[6] with bone fragility persisting throughout life. Individuals with mild forms of the disease may have normal stature with no deformities or fractures at all, and the condition would be diagnosed only when an x-ray is obtained for other reasons. People with severe OI may have extreme short stature and severe deformity of the long bones. Exercise tolerance and muscle strength are significantly reduced in patients with OI, even in the mild forms.[7]

Osteogenesis imperfecta can affect several organs and systems. For example, hearing loss may be present in about 50% of the

From The Editor's Desk

Dear Colleague:

You may be aware that our former sponsor, Insmed, settled a patent infringement dispute and no longer promotes IGF-I/IGFBP-3 to patients with severe primary IGF-I deficiency or other short stature indications. Therefore, they no longer provide an educational grant to the GGH journal. Consequently, Pediatric Sunshine Academics, Inc., a 501(c)(3) non-profit organization, is funding the cost of this issue of GGH without prior anticipation or alternative funding sources available.

However, I am committed to seek new grants that will allow us to continue publishing this journal. I am grateful to the editorial board for their strong support; they have all pledged to contribute with their usual efforts and expertise while we seek more stable times. Since its inception 23 years ago, GGH has improved and expanded; it is held in high regard and enjoys over 11 000 subscribers. We all feel obliged not to let you down.

In order to forge ahead GGH will need the support of its readers while we elicit educational grants. You can help us during this transition by contributing to Pediatric Sunshine Academics, Inc. an organization whose mission is to support research and education in pediatric endocrinology and nutrition. Your fully tax deductible donation to **Pediatric Sunshine Academics, Inc., P. O. Box 3208, Tallahassee, FL 32315-3208**, either by check or online at **www.PedSacademics.org** will be used entirely for the continued publication of GGH. Pediatric Sunshine Academics, Inc.'s federal EIN is 65-0854085.

On behalf of the editorial board, I thank you in advance for your donations and support. I will keep you apprised of our quest to elicit new grants and sponsorships for the continuation of the publication of GGH.

Fima Lifshitz, MD
Editor-in-Chief

GGH is supported by an unrestricted educational grant from Pediatric Sunshine Academics

individuals with mild forms of OI after the third decade of life.[8] The incidence of congenital malformations of the heart in children with OI is probably similar to that of the normal population,[9,10] but respiratory complications secondary to kyphoscoliosis are common in individuals with severe OI.[11] Joint hyperlaxity is also a common occurrence in patients with OI,[12] and may lead to dislocation of hips and radial heads, sprains, and flat feet. Constipation and hernias are also a common complication of OI.[13] Dentinogenesis imperfecta (DI), caused by an abnormal dentin while enamel remains normal,[14,15] is prevalent in about 28% of OI patients.[16] Life expectancy in subjects with non-lethal OI appears to be the same as that in the normal population,[17] with the exception found in cases of very severe OI with respiratory or neurological complications.[18]

Histomorphometric analysis of the bone in patients with OI shows decreased trabecular bone volume, possibly secondary to the formation of fewer trabeculae, and to a lack of thickening of trabeculae with growth. There is evidence of defects in modeling of external bone size and shape, production of secondary trabeculae by endochondral ossification, and thickening of secondary trabeculae by remodeling.[19] Contrary to the common conception of attributing the defect in OI to the osteoclast, OI should be regarded as a disease of the osteoblast. Collagen plays an essential role in forming an interactive network between the cells by making extracellular matrix and noncollagenous proteins that lead to proper mineralization of the bone. When the fundamental structure of the collagen helix is disturbed by a mutation, a complex series of secondary changes to the bone develops, leading to increased bone fragility.

GROWTH IN CHILDREN WITH OI

Severely affected patients may be short because of vertebral compression fractures, severe scoliosis, lower limb deformities, and disruption of growth plates.[20] However, growth can also be delayed in the absence of these abnormalities. The most commonly used classification divides OI into 4 types. Type I patients do not have bone deformities and may have normal height, but fractures may range from very few to dozens over a lifetime. Type II is the most severe, with patients usually not surviving the perinatal period. Patients with type III have a characteristic triangular face, very short stature, and severe bowing of long bones; they typically suffer many fractures throughout their life. Type IV is not clearly defined. Patients with this type of OI are generally short, although there is no consensus regarding the specific characteristics of this type. Other types have been described, but there is controversy because they actually represent syndromes resembling OI.[1] According to one study, during the first 10 years of life the number of fractures, extent of skeletal deformities, and growth retardation do not differ between OI types III and IV.[10] This is surprising, as individuals with type III OI usually have very short stature, whereas individuals with type IV OI may have mild-to-moderate short stature. Furthermore, according to some authors, individuals with type IV OI may have normal stature.[21] This highlights the inaccuracy of classifying this disease into 4 types.[22] I will, therefore, refer to OI "severity" throughout this article, instead of OI "type."

The mean standing height of patients with OI is lower than that of their unaffected first degree family members, regardless of severity. Truncal height is reduced and head size increased in one third of the patients, more so in individuals with moderate or severe OI (Sillence's types IV and III). During childhood, there appears to be no difference between the standing heights of girls and boys, but women had lower height z-scores than men. The reduction in arm span z-score generally follows the same pattern as for height: individuals with moderate or severe OI tend to have lower z-scores than individuals with mild OI. The arm span/height ratio appears to be increased in children with moderate or severe OI, but not in those with mild OI. Mean concentrations of insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 are generally normal, in the low range of age-specific reference values.[10,21] Growth hormone (GH) deficiency is very rare in patients with OI. In a group of 22 children tested by Marini et al,[23] none fulfilled the standard criteria for GH deficiency. A few children in that study had a blunted response to GH-releasing hormone or failed to double their serum IGF-I in a 5-day somatomedin generation test. However, there was no consistent relationship between those responses or between the responses and type of OI.

The etiology of the growth restriction in children with moderate and severe OI is not entirely clear. It has been suggested that it could be viewed as a self-protective mechanism: a given mechanical load creates smaller stresses in a short bone than in a long bone, thus a short bone will break less easily.[24] People with severe OI have a typical deformity of the growth cartilage, defined as "popcorn" appearance of the metaphysis. Microfractures of the growth cartilage may play a role in the growth problems experienced by these patients. There are no reports on the effects of puberty and hormonal changes on growth in children with OI.

USE OF BISPHOSPHONATES IN CHILDREN WITH OI

Bisphosphonates are synthetic drugs with a chemical structure based on pyrophosphate,[25] and have been used to treat osteopenia of primary and secondary origin in both children and adults.[26] Effects on both osteoblasts[27,28] and osteoclasts[29,30] have been shown, although the mechanism through which bisphosphonates increase bone mineral density (BMD) is not clear (Figure 1). Likewise, effects of bisphosphonates on growth have been documented, but the mechanism of those effects has not been elucidated. There are differences

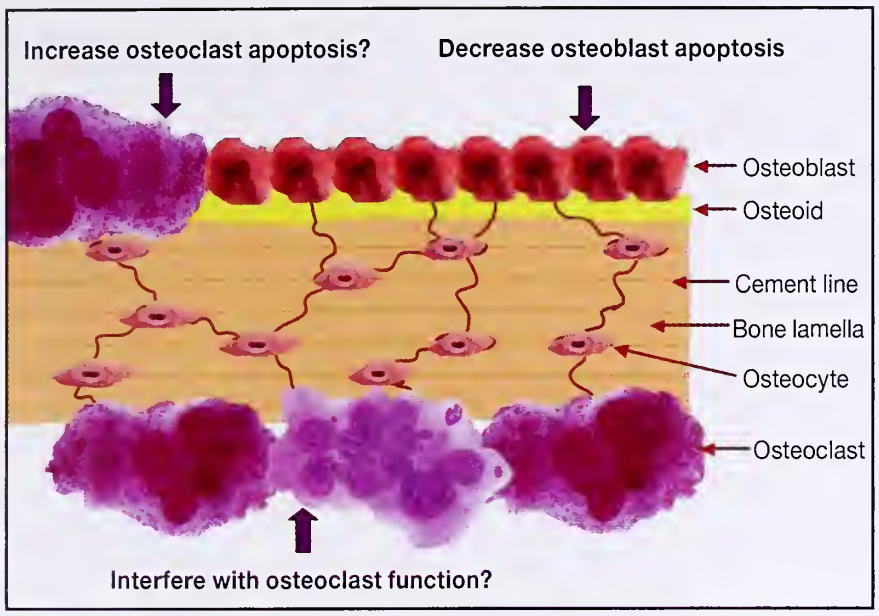

Figure 1. Possible mechanisms of action of bisphosphonates on bone.

among the bisphosphonates that may influence their mechanisms for binding and inhibiting bone crystal growth and dissolution. This may explain differences in potency among different bisphosphonates, such as the apparently more prolonged duration of action of alendronate and zoledronic acid, compared with the more readily reversible effects of risedronate.[31]

Different treatment protocols recommend the use of different bisphosphonates (ie, pamidronate, risedronate, alendronate, olpadronate, neridronate), and at different dose regimens for the pediatric population. For example, pamidronate doses range from 4.5 mg/kg/yr[32,33] (Tables 1 and 2) to 9 mg/kg/yr.[34,35] Children treated with high-dose pamidronate experience dramatic increase in BMD, with changes of as high as 200% per year.[35,36] Other positive effects observed include increase of the cortical width of the metacarpals, and increased vertebral height in previously fractured vertebrae. The incidence of fractures decreases as well. Fracture healing does not appear to be impaired in patients with OI when compared to untreated OI patients.[37,38] There is a striking disappearance of bone pain and decreased fracture incidence noted with intravenous treatment. This may contribute to greater mobility,[39,40] an essential factor for the development of the skeletal system.[41] A lower fracture incidence, despite higher risk of injury due to increased mobility, suggests a direct effect of the therapy. These effects contribute to an improvement in the quality of life of patients with OI who are receiving treatment.

Table 1. Protocol for administration of low-dose IV pamidronate treatment.

Age group	Dose	Interval
<2.0 years	0.37 mg/kg/day for 2 days	2 months
2.0-3.0 years	0.56 mg/kg/day for 2 days	3 months
>3.0 years	0.75 mg/kg/day for 2 days	4 months

Table 2. Suggested dilution and infusion rates for IV pamidronate treatment.

mg of pamidronate	mL of normal saline	mL/hr
0-5	50	15
5.1-10	100	30
10.1-15	150	45
15.1-25	250	75
25.1-45	500	150

A side effect of high doses of pamidronate (9 mg/kg/yr) is retention of calcified cartilage within secondary spongiosa in children with OI.[30] Higher doses have caused osteopetrosis in a patient with no diagnosis.[42] Retention of calcified cartilage within secondary spongiosa is a hallmark of osteopetrosis, this suggests a dose-related effect of pamidronate. Studies using oral bisphosphonates for the treatment of OI (olpadronate,[43] alendronate) showed no differences between the drugs and placebo on functional outcome, anthropometrics, fracture incidence, or vertebral height, although it has been suggested that oral alendronate may improve quality of life in this group of patients.[44]

EFFECTS OF BISPHOSPHONATE TREATMENT ON GROWTH IN CHILDREN WITH OI

The effect of treatment with bisphosphonates on longitudinal bone growth in children has been a concern among clinicians.[45] Bone resorption is an essential part of the normal endochondral ossification process,[46] and of the bone modeling and remodeling process. Despite the fact that the mechanism of long bone growth relies upon clonal expansion and subsequent hypertrophy of chondrocytes, endochondral bone growth requires resorption of the septa of calcified cartilage at the chondro-osseous junction of the growth plate by chondroclasts, permitting vascular invasion of the hypertrophic cell lacunae.[47] Drugs interfering with this mechanism could potentially cause impairment of the bone elongation process. Bisphosphonates interfere with osteoclast function[29] or survival,[48] and could, therefore, have a deleterious effect on bone growth. This undesired effect has actually been shown in animal studies.[49] High doses of alendronate (>2.5 mg/kg/wk) inhibited long bone length in the OIM mice (a model of OI) through alteration of the growth plate and possibly reduced resorption at the chondro-osseous junction.[50] Furthermore, lower doses of alendronate do not appear to have a detrimental effect on growth in oim/oim mice,[51] suggesting another dose-related effect of bisphosphonates. On the other hand, bisphosphonates do not appear to be detrimental for growth in human subjects at the doses currently used (Figure 2).[24,52-55] Each time a patient receives a pamidronate infusion, a new sclerotic line appears in the

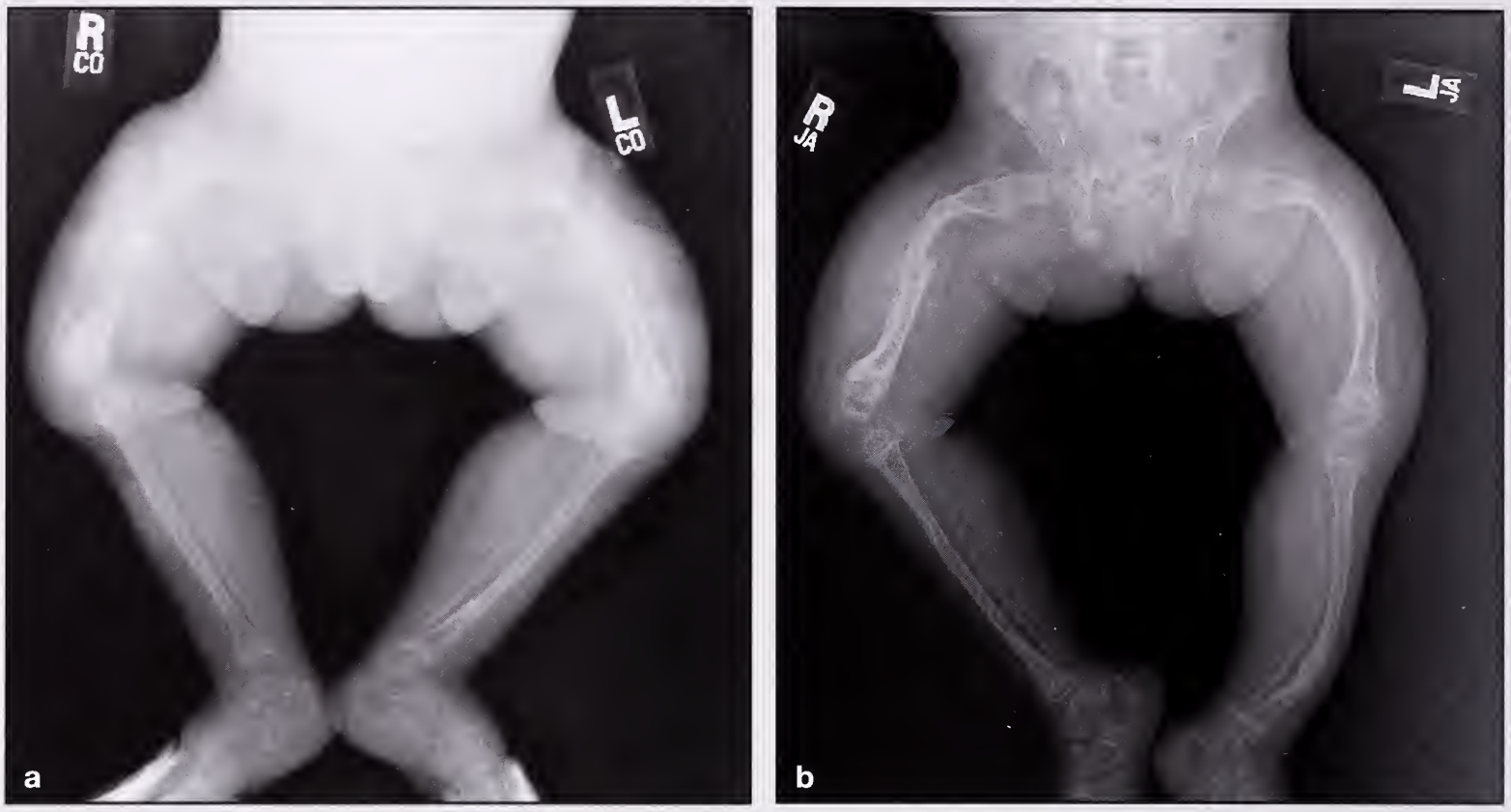

Figure 2. Lower limbs x-ray of a child with severe OI before (a) and after 12 months (b) of treatment with low-dose pamidronate (4.5 mg/kg/yr). Treatment was started at 18 months of age. Note longitudinal growth. Fractures do occur under treatment as evident in the panel on the right, but at much lower rate than before treatment.

metaphysis of long bones. The distance between these lines reflects longitudinal bone growth (Figure 3).

Pamidronate in high doses (9 mg/kg/yr) does not appear to negatively affect growth. Height z-score actually increased in a group of patients with OI who had started treatment before 3 years of age.[35] After one year of pamidronate therapy, height z-scores increased significantly in a group of children with severe OI and did not change in children with mild and moderate OI.[24] After 4 years of therapy with the same dose regimen of pamidronate, mean height z-scores increased significantly in children with moderate OI, whereas non-significant trends to increase were seen in patients with mild and severe OI.[24] Low doses of pamidronate appear to have a similar effect (data not published) (Figure 3).

Low doses of pamidronate elicited no short-term evidence of growth impairment in children with a variety of pathologies leading to osteoporosis, including OI. A median annualized change in height SDS of 0 (range, –0.4 to 0.5) was noted in that group.[56] As expected, growth changes are greater in children with milder OI than in those with more severe forms of the condition when receiving therapy with alendronate or pamidronate.[57]

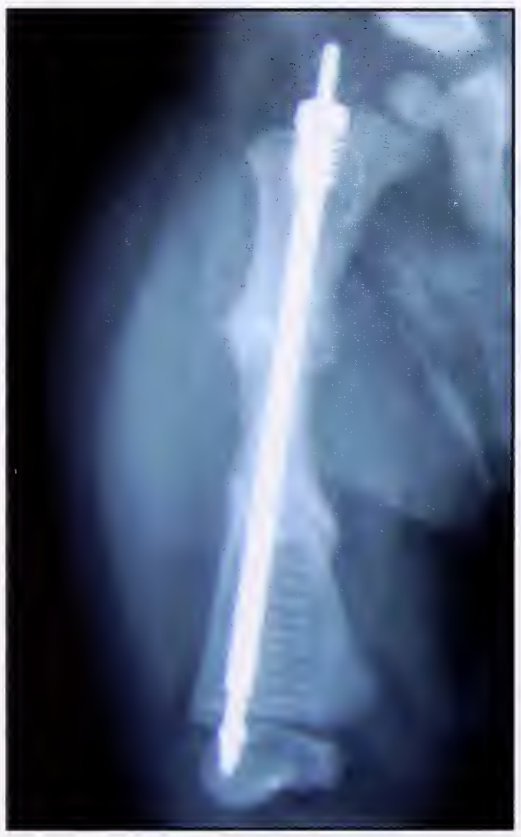

Figure 3. Distal femur of a pediatric patient with OI receiving treatment with IV pamidronate. Note the sclerotic lines, each representing an infusion. The distance between lines reflects longitudinal bone growth in a 2-month period.

One study showed that patients treated with high doses of pamidronate (9 mg/kg/yr) had similar growth plate width but wider metaphyses when compared with untreated OI patients who were matched for OI type and age, despite the lack of detrimental effects of bisphosphonates on longitudinal growth,[58] suggesting an effect of the high dose on bone remodeling. A different study showed that metaphyseal modeling in the distal femur is constant in children on bisphosphonates—with slight variation between sexes—resulting in a similar shape of the distal femur throughout childhood when looking at the modeling process.[59] Noteworthy, the observed positive effect of pamidronate on bone growth does not appear to be secondary to acceleration of bone age.[24]

Infants with OI appear to grow better when treatment with neridronate is started soon after birth, rather than at 6 months of age.[60] Older children with OI receiving neridronate grew faster than controls in one study.[61] At the microscopic level, the size of iliac crest bone biopsies is not significantly different before or after treatment in children

with OI. Changes are seen in cortical width, which increased by about 90%. Cancellous bone volume increases by about 45% with treatment. This change is due to higher trabecular number, with no change in trabecular thickness.[30] Importantly, there is no evidence for a mineralization defect in children with OI treated with high doses of pamidronate.[30] Growth in children continues after treatment with pamidronate is stopped, and the newly-formed bone will be unprotected and prone to fractures (Figure 4).

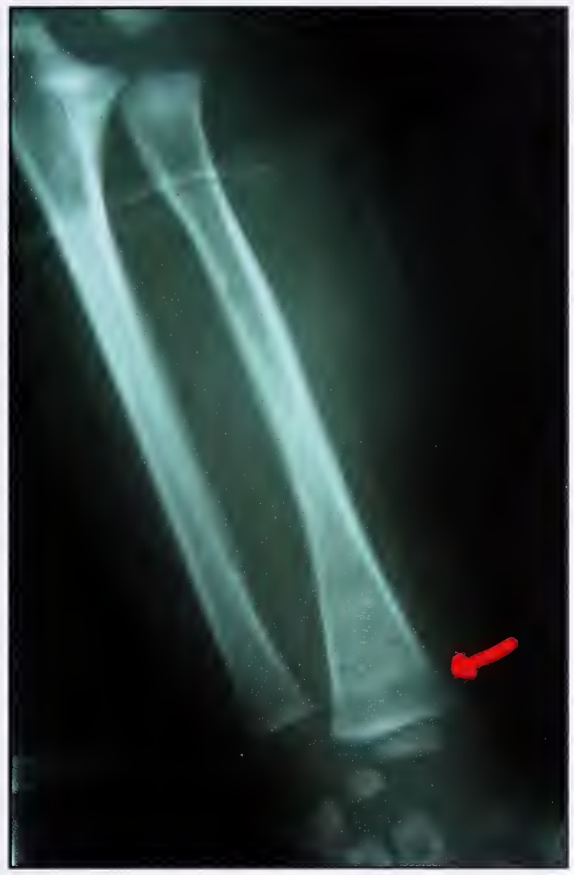

Figure 4. Long bones continue growing after treatment with bisphosphonates is stopped (arrow), causing susceptibility to fractures.

LONG-TERM EFFECTS OF BISPHOSPHONATES ON HEIGHT

In one study, mean height z-scores of subjects with all OI degrees of severity tended to increase after 4 years of pamidronate therapy when compared with baseline. However, the change in height z-scores was significant only for the group with moderate OI, but not for mild or severe OI.[24] It is of note that these comparisons were done against normal growth charts designed for healthy children. To more accurately assess the growth rate of children with OI undergoing treatment, the same group compared their growth with that of a group of children with OI who were not receiving treatment with bisphosphonates. In that study, each height measurement of patients was expressed as a percentage of the mean value expected for untreated OI patients. During 4 years of pamidronate therapy, height significantly increased above the values expected for untreated patients.[24]

EFFECT OF PAMIDRONATE ON FINAL HEIGHT

There is very little information about final height in children with OI treated with bisphosphonates. There is the description of only 8 patients who attained final height while receiving treatment with pamidronate.[24] In this study, final height, expressed as a percentage of the expected height in untreated patients, was significantly higher than baseline height. This study suggests that an average gain of 7 cm in patients with mild OI, 12 cm in patients with moderate OI, and 9 cm in patients with severe OI can be expected at 15 years of age. These results suggest that acceleration of growth is not just a transitory effect, but rather a lasting outcome on height in children with OI who are receiving pamidronate intravenously. As mentioned above, it is not entirely clear how pamidronate treatment might improve growth. Part of the effects of bisphosphonates on growth in children with moderate and severe OI could be due to prevention of long bone deformity and regeneration of vertebral fractures (Figure 5),[35,62] and to prevention of microfractures affecting growth cartilage.

EFFECTS OF GROWTH HORMONE IN CHILDREN WITH OI

Growth hormone regulates post-natal bone growth; IGF-I mediates the growth-promoting action of GH, although it has been shown that GH may have independent, direct effects on growth.[63] Also, IGF-I has mitogenic effects in dividing cells and is closely associated with growth, although plasma levels do not correlate with growth rates. It is known to

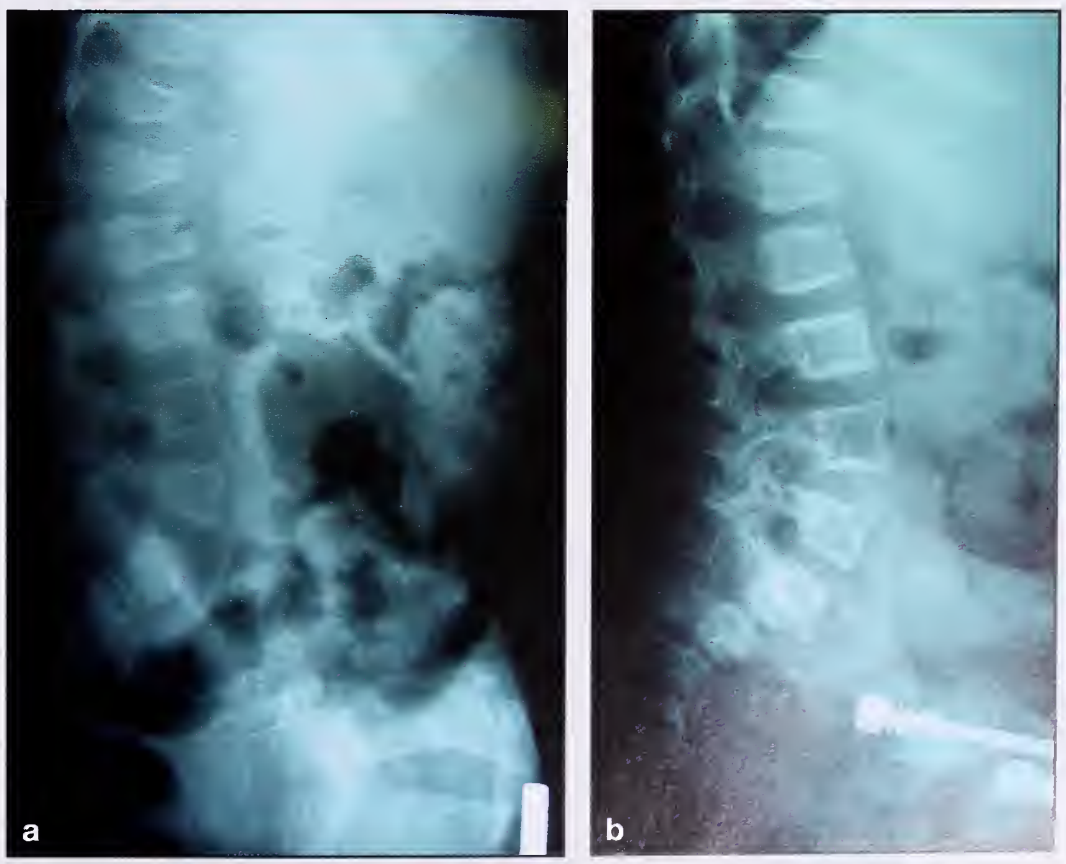

Figure 5. Lumbar spine x-ray of a child with severe OI before (a) and after 12 months (b) of treatment with low-dose pamidronate (4.5 mg/kg/yr). Treatment was started at 18 months of age. Note increased vertebral height with treatment.

increase 1-α hydroxylase in kidneys, with subsequent increased production of calcitriol (1,25 [OH]2 vitamin D3). As calcitriol is the active form of vitamin D, IGF-I and GH treatment can make calcium more available for bone mineralization,[64] which could add to a possible beneficial effect in patients with OI. Inversely, it has been suggested that vitamin D, calcium, and protein supplements may elicit part of their effect on osteoporosis through increased IGF-I levels.[65]

IGF-I promotes longitudinal bone growth by 'insulin-like' anabolic actions which augment chondrocyte hypertrophy.[66] Chondrocyte differentiation, in turn, leads to cartilage expansion and linear growth. Furthermore, osteoblasts and pre-osteoblasts secrete IGF-I, and bone resorption causes release of stored IGF-I. This hormone appears to be a growth factor for osteoblasts. A homozygous molecular defect in the gene encoding IGF-I caused severe intrauterine growth failure, sensorineural deafness, and mild mental retardation in one individual.[67] Treatment with IGF-I improved linear growth and insulin sensitivity in that patient.[68] There is also some weak evidence that IGF-I has a role in declining BMD with aging. In patients with Laron syndrome, IGF-I treatment increases bone growth in the absence of GH.[69] Low IGF-I concentrations appear to be associated with low BMD in patients with cystic fibrosis.[70]

As discussed above, some children with OI have a blunted response to GH-releasing hormone or fail to double their serum IGF-I in a 5-day somatomedin generation test (13 of 22 had less than a 2-fold stimulation of somatomedin-C by GH).[71] There was no overlap between the group with blunted IGF-I response and the group with decreased GH-releasing hormone response, suggesting that there might be 2 different mechanisms of GH resistance in children with OI. GH is an anabolic hormone and, together with IGF-I, is a potent regulator of muscle mass. As such, there is potential for it to increase bone density. In the absence of trauma, muscles are responsible for the largest loads and the largest bone strains, and those strains help to control the biological mechanisms that determine whole-bone strength (Figure 6).[72]

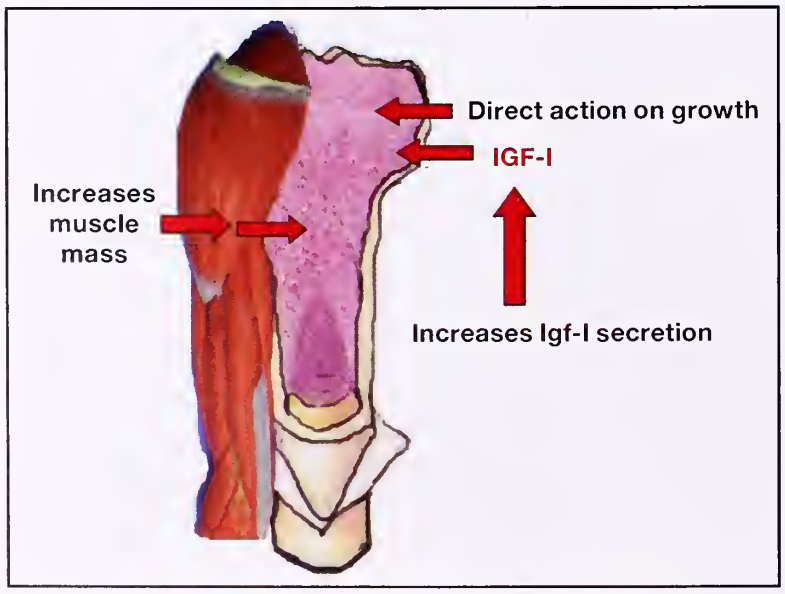

Figure 6. Mechanisms for growth hormone stimulation of bone growth and increase of bone mineral density.

There are no large controlled studies of GH treatment in children with OI. Furthermore, there are no data in the literature regarding final height in OI patients treated with GH. An increase of fracture rate during GH therapy has been reported in children with OI by different groups,[73,74] although another group did not find an increase in fracture rate in a small group of children with mild OI who were treated with GH for 1 year.[75] Extending treatment to 2 years did not change the fracture risk either.[76] Like all children who are initially started on GH, children with OI experience an initial acceleration of growth rate,[64,77] but a sustained effect has not been demonstrated. In one study, GH (0.1- 0.2 IU/kg/d for 6 days/wk) was administrated for at least 1 year to children with OI of different severity;[78] about one-half of the treated OI children sustained a 50% or more increase in linear growth, compared to their baseline growth rate. It is of note that most responders (10 of 14) did not have a severe form of OI. Incidentally, only the linear growth responders had a significant decrease in long bone fractures. After 1 year of treatment, responders' iliac crest biopsy showed significant increases in cancellous bone volume, trabecular number, and bone formation rate, but no significant increase in cortical thickness. Histomorphometric parameters of bone resorption were not significantly changed in responders, whereas non-responders had an 80% increase in the percentage of bone surface covered by osteoclasts. The incidence of fractures was unchanged in non-responders. Bone formation parameters did not increase with treatment in this group. Although progression of scoliosis was unchanged compared with the National Institute of Child Health and Human Development (NICHD) OI population, data on individual cases are not offered in the report.

Recombinant human IGF-I, complexed with its predominant binding protein IGFBP-3 is currently being tested as a treatment for osteoporosis, alone or in combination with anti-resorptive drugs and GH.[79] There appears to be a correlation between the dose of GH (and the obtained IGF-I plasma levels) and the increase in bone turnover markers and/or BMD in adults,[80] although a different study found that 1 year of IGF-I treatment, at a dose sufficient to elevate circulating IGF-I to young normal values, was not an effective means to alter body composition or blood parameters, nor to improve bone density, strength, mood, or memory in older women.[81]

CONCLUSION

Bisphosphonate treatment does not appear to have a detrimental effect on linear growth in children and adolescents with OI, regardless of the severity of the condition. Long-term bisphosphonate therapy in children with OI may be associated with a significant height gain, as compared with untreated OI patients with the same

disease severity. The use of GH in this population is still controversial. It has been suggested that GH treatment should probably not be used as a first-line therapy in OI.[82] Combined protocols administering both bisphosphonates and GH are warranted. Other therapeutic options currently used or in research for patients with osteoporosis (PTH, IGF-I, strontium, RANK ligand) may have a role in the treatment of OI in the future.

Disclosures: The author discloses no conflicts of interest. Pamidronate use for children with OI is off label.

References

1. Plotkin H. BMC Pediatr. 2004;4:16.
2. Dalgleish R. Nucleic Acids Res. 1997;25:181-7.
3. Orioli IM, Castilla EE, Barbosa-Neto JG. J Med Genet. 1986;23:328-32.
4. Smith R. Int J Exp Pathol. 1994;75:233-41.
5. Lund AM, Schwartz M, Skovby F. Clin Genet. 1996;50:304-9.
6. Albright JA, Grunt JA. J Bone Joint Surg Am. 1971;53:1415-25.
7. Takken T, Terlingen HC, Helders PJ, et al. J Pediatr. 2004;145:813-8.
8. Garretsen AJ, Cremers CW, Huygen PL. Ann Otol Rhinol Laryngol. 1997;106:575-82.
9. Hortrop J, Tsipouras P, Hanley JA, et al. Circulation. 1986;73:54-61.
10. Vetter U, Pontz B, Zauner E, et al. Calcif Tissue Int. 1992;50:36-41.
11. Falvo KA, Klain DB, Krauss AN, et al. Am Rev Respir Dis. 1973;108:1258-60.
12. Wordsworth P, Ogilvie D, Smith R, et al. Br J Rheumatol. 1987;26:9-12.
13. Spence PA, Cohen Z, Salerno TA. Can Med Assoc J. 1984;134:1369-70.
14. Lukinmaa PL, Ranta H, Ranta K, et al. J Craniofac Genet Dev Biol. 1987;7:115-25.
15. Lindau BM, Dietz W, Hoyer I, et al. Int J Paediatr Dent. 1999;9:13-21.
16. Lund AM, Jensen BL, Nielsen LA, et al. J Craniofac Gen Dev Biol. 1998;18:30-7.
17. Paterson CR, Ogston SA, Henry RM. BMJ. 1996;312:351.
18. McAllion SJ, Paterson CR. J Clin Pathol. 1996;49:627-30.
19. Rauch F, Travers R, Parfitt AM, et al. Bone. 2000;26:581-9.
20. Plotkin H. Syndromes with brittle bones, hyperostotic bone diseases and fibrous dysplasia of bone. In: Lifshitz F, ed. Pediatric Endocrinology. Vol 2, 5th edition. New York, NY: Informa;2007:559-70.
21. Lund AM, Muller J, Skovby F. Arch Dis Child. 1999;80:524-8.
22. Plotkin H. J Ped Orthop. 2006;26:148-9.
23. Marini JC, Bordenick S, Heavner G, et al. Am J Med Genet. 1993;45:261-4.
24. Zeitlin L, Rauch F, Plotkin H, et al. Pediatrics. 2003;111:1030-6.
25. Rodan GA, Balena R. Ann Med. 1993;25:373-8.
26. Orcel P, Beaudreuil J. Joint Bone Spine. 2002;69:19-27.
27. Plotkin LI, Weinstein RS, Parfitt AM, et al. J Clin Invest. 1999;104:1363-74.
28. Mathov I, Plotkin LI, Sgarlata CL, et al. J Bone Miner Res. 2001;16:2050-5.
29. Halasy-Nagy JM, Rodan GA, Reszka AA. Bone. 2001;29:553-9.
30. Rauch F, Travers R, Plotkin H, et al. J Clin Invest. 2002;110:1293-9.
31. Nancollas GH, Tang R, Phipps RJ, et al. Bone. 2006;38:617-27.
32. Plotkin H, Coughlin S, Kreikemeier R, et al. Dev Med Child Neurol. 2006;48:709-12.
33. Gandrud LM, Cheung JC, Daniels MW, et al. J Pediatr Endocrinol Metab. 2003;16:887-92.
34. Glorieux FH, Bishop NJ, Plotkin H, et al. N Engl J Med. 1998;339:947-52.
35. Plotkin H, Rauch F, Bishop N, et al. J Clin Endocrinol Metab. 2000;85:1846-50.
36. Munns CF, Rauch F, Travers R, et al. J Bone Miner Res. 2005;20:1235-43.
37. Munns CF, Rauch F, Zeitlin L, et al. J Bone Miner Res. 2004;19:1779-86.
38. Pizones J, Plotkin H, Parra-Garcia JI, et al. J Pediatr Orthop. 2005;25:332-5.
39. Plotkin H, Gibis J, Glorieux FH. Bone. 2001;28:S78.
40. Montpetit K, Plotkin H, Rauch F, et al. Pediatrics. 2003;111: 601-3.
41. Frost HM. Calcif Tissue Int. 1988;42:145-56.
42. Whyte MP, Wenkert D, Clements KL, et al. N Engl J Med. 2003;349:457-63.
43. Sakkers R, Kok D, Engelbert R, et al. Lancet. 2004;363:1427-31.
44. Seikaly MG, Kopanat IS, Salhab N, et al. J Pediatr Orthop. 2005;25:786-91.
45. Srivastava T, Alon US. Clin Pediatr (Phila). 1999;38:687-702.
46. Ross FP. Osteoclast biology and bone resorption. In: Favus MJ, ed. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 6 ed. Washington, DC: ASBMR; 2006:30-5.
47. Farnum CE, Wilsman NJ. J Orthop Res. 1989;7:654-66.
48. Hughes DE, Wright KR, Uy HL, et al. J Bone Miner Res. 1995;10:1478-87.
49. Pataki A, Muller JR, Green JR, et al. Anat Rec. 1998;249:458-68.
50. Evans KD, Lau ST, Oberbauer AM, et al. Bone. 2003;32:268-74.
51. McCarthy EA, Raggio C, Hossack M, et al. Pediatr Res. 2002;52:660-70.
52. Lee YS, Low SL, Lim LA, et al. Eur J Pediatr. 2001;160:641-4.
53. Zacharin M, Bateman J. J Pediatr Endocrinol Metab. 2002;15:163-74.
54. Forin V, Arabi A, Guigonis V, et al. Joint Bone Spine. 2005;72:313-8.
55. Vallo A, Rodriguez-Leyva F, Rodriguez Soriano J. Acta Paediatr. 2006;95:332-9.
56. Steelman J, Zeitler P. J Pediatr. 2003;142:417-23.
57. DiMeglio LA, Peacock M. J Bone Miner Res. 2006;21:132-40.
58. Land C, Rauch F, Glorieux FH. J Bone Miner Res. 2006;21:374-9.
59. Ward K, Cowell CT, Little DG. Bone. 2005;36:999-1002.
60. Antoniazzi F, Zamboni G, Lauriola S, et al. J Pediatr. 2006;149:174-9.
61. Gatti D, Antoniazzi F, Prizzi R, et al. J Bone Miner Res. 2005;20:758-63.
62. Astrom E, Soderhall S. Arch Dis Child. 2002;86:356-64.
63. Lupu F, Terwilliger JD, Lee K, et al. Dev Biol. 2001;229:141-62.
64. Vieira NE, Goans RE, Weiss GH, et al. Calcif Tissue Int. 2000;67:97-100.
65. Schurch MA, Rizzoli R, Slosman D, et al. Ann Intern Med. 1998;128:801-9.
66. Jie W, Jian Z, Bondy C. FASEB J. 1999;13:1985-90.
67. Woods KA, Camacho-Hubner C, Barter D, et al. Acta Paediatr Suppl. 1997;423:39-45.
68. Camacho-Hubner C, Woods KA, Miraki-Moud F, et al. Growth Horm IGF Res. 1999;9:47-51.
69. Laron Z. Pediatr Endocrinol Rev. 2004;2:78-85.
70. Gordon CM, Binello E, LeBoff MS, et al. Osteoporos Int. 2006;17:783-90.
71. Marini JC, Bordenick S, Heavner G, et al. J Clin Endocrinol Metab. 1993;76:251-6.
72. Frost HM, Schonau E. J Pediatr Endocrinol Metab. 2000;13:571-90.
73. Kinugasa A, Fujita K, Inoue F, et al. Acta Ped Scan. 1991;379:559-60.
74. Kodama H, Kubota K, Abe T. J Pediatr. 1998;132:559-60.
75. Antoniazzi F, Bertoldo F, Mottes M, et al. J Pediatr. 1996;129:432-9.
76. Antoniazzi F. Therapeutic approach with growth hormone. In: 7th International Conference on Osteogenesis Imperfecta. Montreal, Canada; 1999:1-16.
77. Vieira NE, Marini JC, Hopkins E, et al. Bone. 1999;25:501-5.
78. Marini JC, Hopkins E, Glorieux FH, et al. J Bone Miner Res. 2003;18:237-43.
79. Boonen S, Rosen C, Bouillon R, et al. J Clin Endocrinol Metab. 2002;87:1593-9.
80. Agnusdei D, Gentilella R. J Endocrinol Invest. 2005;28:32-6.
81. Friedlander AL, Butterfield GE, Moynihan S, et al. J Clin Endoc Metab. 2001;86:1496-503.
82. Wright NM. J Ped Endocrinol Metab. 2000;13:999-1002.

REVIEWS & COMMENTS FROM THE LITERATURE

Childhood Hypopituitarism after Traumatic Brain Injury

The hypothalamus and pituitary are essential for childhood and adolescent development and are vulnerable to injury and dysfunction following brain trauma. Hypothalamic-pituitary dysfunction has been well recognized after traumatic brain injury (TBI) in adults. However, data regarding hypothalamic-pituitary function in brain-injured children and adolescents are scant. It is necessary for physicians as well as patients and family members to know that onset of hypothalamic-pituitary deficits can occur even after several years following brain injury.

Acerini et al reviewed the available pediatric data, which showed that after both mild and severe TBI, hypopituitarism may occur; growth hormone (GH) and gonadotropin deficiencies appear to be most common. Precocious puberty has also been documented. Detailed investigations of pituitary function have been reported in 20 patients (12 males, 7 females, and 1 sex unspecified). Subjects ranged in age from infancy to 16 years at the time of injury; they were investigated between 1 and 42 years after the initial episodes of TBI. All patients had multiple anterior pituitary hormone deficiencies, except one, who had isolated GH deficiency. The frequencies of deficient hormones were: GH 85%, LH/FSH 80%, TSH 75%, and ACTH 50%. It was notable that in 6 patients, multiple deficiencies were documented after relatively mild head injury without loss of consciousness. Pituitary stalk transaction was demonstrated on MRI in several cases. The diagnosis of hypothalamic-pituitary deficiency was made during childhood and adolescence in 17 of the 20 patients and during adult life in the remaining 3. The key presenting symptoms were growth failure, delayed or arrested puberty, secondary amenorrhea or reduced libido. Delay in the diagnosis was extreme in many cases and hypopituitarism was clearly not considered as a possible complication of the TBI until defects of growth or reproductive function became obvious.

Acerini and colleagues urged pediatric endocrinologists, in collaboration with adult endocrinologists, to perform formal prospective research studies in patients suffering from TBI to clarify prevalence, natural history, and response to hormone replacement.

Acerini CL, Tasker RC, Bellone S, Bona G, Thompson CJ, Savage MO. Hypopituitarism in childhood and adolescence following traumatic brain injury: the case for prospective endocrine investigation. Eur J Endocrinol. 2006;155:663-9.

First Editor's Comment: *This is a very interesting report which provides important information for physicians who care for patients with TBI. Traumatic brain injury is a worldwide health problem and a major leading cause of death and disability among young adults. Survivors are often left with significant neuroendocrine dysfunction and adverse physical and/or psychological problems which are perhaps an even greater risk than previously considered. As well, TBI-induced hypopituitarism has been under-recognized, under-investigated, and untreated. Relatively little attention has been paid to the possibility of TBI-induced hypopituitarism, especially in children. As reported by Acerini et al, it became clear that TBI posed substantial risk to hypothalamic-pituitary function in children; the onset of hypopituitarism can evolve over years following injury.*

Road-traffic accidents, falls, sports injuries, and child abuse are the most common etiological factors for pediatric TBI, although the causes are different among age groups. The perinatal brain injury such as difficult forceps delivery at breech delivery is a well-known cause of hypopituitarism. Infants with TBI have primarily suffered from falls or assaults. Toddlers are more frequently injured as passengers in motor vehicle accidents, although falls still account for the majority of injuries. Children and infants have large, heavy heads with weaker cervical ligaments and muscles compared to adults. Given the same deceleration of the body, head trauma is therefore more likely in infants and younger children than adults. Similarly, the resulting brain injury may be more severe due to the thin, pliable skull and unfused sutures of infants and young children. Possible causes of hypopituitarism include hemorrhage, infarction, ischemia, swelling, stalk transection, or direct trauma to the hypothalamus, stalk, and/or pituitary region. Severity of TBI seems to be an important risk factor for developing hypopituitarism; however, even mild trauma may precede hypopituitarism. Accurate evaluation and long-term follow-up of all TBI patients are necessary in order to detect the occurrence of hypopituitarism, regardless of clinical evidence for pituitary dysfunction. The most common endocrine alterations appear to be GH and gonadotropin, followed by ACTH and TSH deficiency. Hyperprolactinemia may also be present. Diabetes insipidus may be frequent in the early, acute phase post-TBI, but it is rarely permanent.

The signs and symptoms of TBI-induced hypopituitarism are often nonspecific and can be additionally masked by what has been assumed to be merely the post-traumatic syndrome. These symptoms are likely to be overlooked if endocrine dysfunction is not actively evaluated. Moreover, hormonal deficits may significantly contribute to the chronic disability and the physical, cognitive, health, and social sequel in patients with TBI. Therefore, regular endocrine evaluation and follow-up should be performed throughout life in patients with TBI. In most instances, patients with

TBI are first seen and treated by trauma surgeons and neurosurgeons, and subsequently by rehabilitation specialists; all physicians must be informed about the risks of TBI-induced hypopituitarism. It is important to increase awareness among physicians, patients, and family members of the risks of hypopituitarism and the need for appropriate endocrinological assessment and adequate hormonal replacement therapy, if necessary. Thorough assessment may make it possible to improve the quality of life and enhance the rehabilitation prospects. Lack of awareness of this problem may result in long-term adverse consequences of untreated hypopituitarism for these patients. A close collaboration among neurosurgeons, neurologists, rehabilitation specialists, and endocrinologists is essential to achieve a coordinated approach to the care of patients with TBI.

Yoshikazu Nishi, MD

Second Editor's Comment: *The consensus guidelines on screening for hypopituitarism following TBI for adults[1] was published in 2005. These guidelines may also apply to children and adolescents as the data in the paper by Acerni et al on the development of hypopituitarism following TBI are similar to the reported alterations found in adults. A summary of selected studies was presented in a tabular form in the consensus statement. However, the appropriate diagnosis and treatment of the endocrine alterations should always be accompanied by evidence-based cognitive rehabilitation of those patients; these recommendations for clinical practice were published by Cicerone et al.[2]*

Fima Lifshitz, MD

References

1. Ghigo E, Masel B, Aimaretti G, et al. Brain Inj. 2005;19:711-24.
2. Cicerone KD, Dahlberg C, Kalmar K, et al. Arch Phys Med Rehabil. 2000;81:1596-615.

The Value of Clinical and Radiological Expertise in Mutation Screening

As with many types of genetic disease, it is becoming common for clinical diagnosis of skeletal dysplasias to be confirmed at the DNA level. An issue that often arises is whether or not cases submitted for DNA diagnosis should be evaluated by experts before performing DNA testing. A small study involving multiple epiphyseal dysplasia (MED) reported by Zankl et al suggests that preselection of cases through such evaluation significantly increases the rate of mutation detection. The investigation was carried out under the auspices of the European Skeletal Dysplasia Network (ESDN).

MED is characterized by delayed and irregular ossification of epiphyses and precocious osteoarthritis. It is inherited as a dominant trait in most cases, and mutations have been identified in genes encoding 5 cartilage extracellular matrix proteins including cartilage oligomeric matrix protein (*COMP*), the 3 chains of type IX collagen (*COL9A1*, *COL9A2*, *COL9A3*), and matrilin 3 (MATN3). Mutations of *COMP* are most common. MED is occasionally inherited in a recessive fashion with mutations identified in the gene coding for the diastrophic dysplasia sulfate transporter (DTDST, *SLC26A2*).

The authors first noted that in a recent study *COMP* mutations were detected in only 36% of 58 families with MED in whom the diagnosis was made by the referring physician, usually a clinical geneticist. Since they expected the rate to be higher they suspected that some of the referral diagnoses were incorrect and that the mutation detection rate could be improved by adding an expert evaluation step between referral and DNA diagnosis.

Between September 2003 and February 2005 a panel of experts in the clinical and radiographical aspects of skeletal dysplasias evaluated, before testing, 35 patients with a diagnosis of MED. Of the 35 patients, 24 were considered to have "classical" MED by the experts, 5 possible MED variants, 2 most likely had type II collagenopathy, and 4 patients were considered "unknowns." Genomic DNA was analyzed from 21 of the 29 patients with classical or possible variant MED. Mutations were detected in 13 of the 16 patients with classical MED and one with a possible MED variant. Of the 14 mutations identified, 13 were *COMP* mutations and one involved *MATN3*. A *COL2A1* mutation was subsequently identified in the patient with clinical features of type II collagenopathy. No mutation was detected in 3 patients considered to have classical MED.

When the numbers were tallied, the mutation detection rate was 81% for patients with classical MED and 67% if patients with possible MED variant were included, both substantially higher than the 36% reported previously. The authors concluded that review of clinical and radiographical features by experts prior to DNA testing substantially improves the rate of mutation detection since cases misdiagnosed by non-experts are excluded. The results also confirm that mutations of COMP are responsible for most cases of MED.

Zankl A, Jackson GC, Crettol LM, et al. Preselection of cases through expert clinical and radiological review significantly increases mutation detection rate in multiple epiphyseal dysplasia. Eur J Hum Genet. 2007;15:150-4.

Editor's Comment: *From time to time the skeletal dysplasia community debates the value of clinical and especially radiographical expertise in the diagnosis of skeletal dysplasias. The argument is sometimes made that with DNA diagnosis becoming easily accessible through academic and commercial laboratories and government-sponsored networks such as the ESDN there is no longer a need for special expertise in*

this field. The issue has received special attention in recent years as prominent radiologists with such expertise, ie, the pioneers of this field, have retired faster than young experts have been trained to fill their niche. Accordingly, this paper is very timely since it documents the value of this expertise. Although not discussed in the paper, another issue is the potential cost savings that could be realized from preselection by experts prior to DNA testing.

William A. Horton, MD

Major Determinants of Height Development in Turner Syndrome Patients Treated with Growth Hormone: Analysis of 987 Patients from KIGS

It is well known that growth hormone (GH) treatment during childhood can lead to a higher adult height in girls with Turner syndrome (TS). This is a report of 987 girls treated to adulthood or near adult height with recombinant human GH from the KIGS data base. Approximately 5 600 girls with TS have been entered into this registry. Data analyzed for this publication included 908 subjects who had reached near adult height, defined as age >15 years with a height velocity during the last year on GH < 2.0 cm/year. The subjects had all been prepubertal during the first year of treatment and had been treated for at least 4 years with a minimum of 5 injections per week. Puberty was defined at the time when spontaneous breast development occurred (Tanner stage B2) or when estrogen replacement therapy was initiated. The following variables were summarized: 1) status at birth: weight SD score, length SD score, ponderal index; 2) genetic background: mother's height SD score, father's height SD score, midparental height (MPH) SD score, and karyotype; 3) treatment modality: GH dosage per kg of body weight and per kg of ideal body weight (weight for height), frequency of GH injections, and accumulated years of GH treatment; 4) variables at the start of treatment: age, bone age, height SD score, weight SD score, height SD score minus MPH SD score, the peak GH concentration in serum during stimulation tests; and 5) variables at puberty onset: age, bone age, height SD score, weight SD score, height SD score minus MPH SD score. SD scores were calculated as follows: SD score = (patient's measured value minus mean value for age and sex-matched normal subjects) ÷ SD of the value for age- and sex-matched normal subjects. These independent variables were utilized in multiple regression analyses to determine which contributed to height or change in height between the start of treatment and the achievement of near adult height.

Age at the onset of GH treatment averaged 9.7 years. The average predicted adult height was 146.1 cm. In most cases the initial average GH dose was 0.27 mg/kg/wk delivered in 7 daily injections. Initial height was –2.4 SDS and had reached –1.9 SDS after 1 year of GH therapy. The median gain in height velocity during this first year was 7.4 cm. The age of onset of puberty was 13.5 years and height at this age was 141.8 cm (–1.4 SDS). The overall gain in height from time GH therapy was started was 21.2 cm or 1.5 SDS. Height gain from the start of puberty to near adult height was 9.4 cm. The average age of patients near adult height was 16.9 years with a bone age of 14.5 years. The median height reached was 151 cm, or median gain of 4.9 above the projected height at the time GH treatment was started. The data also showed that height at near adult height was a function of (in order of importance) 1) height at GH start, 2) responsiveness to GH in the first year of treatment, 3) mid-parental height, 4) age at onset of puberty, 5) age at GH start, and 6) mean dose of GH per week. Each of these parameters were significant at a probability level of $p<0.01$ and accounted for 67% of the variance in near adult height.

The gain in height between start of therapy and near adult height was found to be a function of (in order of importance) 1) age at GH start, 2) GH responsiveness during the first year, 3) age at puberty, 4) mid-parental height, 5) height at GH start, 6) mean dose of GH per week, and 7) birth weight. These factors explain 90% of the variance in near adult height. Of note, karyotype did not enter into the multiple regression analyses.

The authors remarked that their observations show that responsiveness to GH during the first year of treatment is the foremost factor in response to height gained during subsequent pre-pubertal years. The data also demonstrated that there is no further gain in relative height after the onset of puberty. They stated that their regression equations support the principal concepts of the current treatment recommendations in TS and further suggest that these equations might be suitable to use as guidelines for daily practice. They cautioned however, that use of such equations might mean that the GH dose and timing at the puberty onset should be adapted to individual patient's responsiveness to GH treatment and that in some cases the GH dose might need to be reduced or discontinued.

Ranke MB, Lindberg A, Ferrández Longás A, et al. Major determinants of height development in Turner syndrome (TS) patients treated with GH: analysis of 987 patients from KIGS. Pediatr Res. 2007:61:105-10.

Editor's Comment: *These data reported from the large KIGS database of girls with TS are both reassuring and disquieting. Clearly GH therapy should be initiated at an early age, regardless of the child's current height. Unfortunately, many girls with TS are not identified until their height falls below the 3rd percentile. The KIGS data suggest that treating these girls at that time while clearly beneficial, is not as beneficial in terms of height gain as treating them shortly after diagnosis regardless of their current height. The data also suggest that higher doses*

of GH, especially during the first year of treatment, may be of significant benefit to achieving greater adult height. Although the authors have carefully developed regression equations for determining near adult height and height gain, between GH start and near adult height, one must be cautioned that statistically significant contributions to the variance in an outcome variable are meant to be used in populations and may not apply in individual cases and may be inappropriate guides for therapy.

William L. Clarke, MD

Focusing Illusion: Wealth, Height, and Happiness

If I were a rich man...: be careful what you wish for. I predict my life would be better if I won the lottery. While I'm pretty happy now, I'd be very happy then. To most of us, that statement seems an obvious truth—not to be questioned, much less explored scientifically. Kahneman (psychologist, winner of the 2002 Nobel Prize in Economics) et al questioned this and the related assumptions using creative research methodologies. They propose a "focusing illusion" is responsible for an exaggeration of the benefits of income to happiness. A focusing illusion occurs when people concentrate attention on the influence of any single factor on their global well-being and exaggerate its importance relative to factors contributing to moment-to-moment happiness.

Evidence for the focusing illusion was found in several lines of research. In one study, students were asked "how happy are you with your life in general" and "how many dates did you have last month." When asked in that order, no correlation ($r = -0.01$) was found; when the statements were reversed, a statistically significant correlation ($r = 0.66$) arose—suggesting that asking about dating exaggerated the salience of that single domain when evaluating one's life on the whole. Another study investigated predicted vs actual effects of several variables on the percentage of time spent in a bad mood. Women were asked what percentage of time they spent in a bad mood yesterday, then to estimate percentages of time spent in a bad mood for people having a lower (<$20k) vs higher (>$100k) income, being alone vs being married for (women >40 years of age) being micromanaged vs not closely supervised at work, and having no health insurance vs excellent benefits at work. Global estimations of bad mood of participants were compared with their own subjective well-being measured moment-to-moment. Predictions for others' mood were compared with actual reports of respondents. The prevalence of bad mood for oneself was overestimated when compared with subjective well-being measured moment-to-moment. Moreover, the prevalence of bad mood predicted for those with less desirable circumstances was grossly exaggerated.

Kahneman et al catalogued several studies providing similar findings and concluded that false intuitions are likely to arise from failure to recognize that people do not continuously think about their circumstances. While recent significant changes in life circumstances (eg, lottery winnings or becoming disabled) may result in multiple daily reflections, an individual's attention eventually returns to the routine (eg, having breakfast or watching TV).

Finding an overall weak relationship between income and happiness or global life satisfaction, Kahneman and colleagues proposed that the focusing illusion helps explain why people seek higher income beyond a modest threshold (predictions exaggerate the increase in happiness) and why the long-term effect of increased income becomes relatively small (attention shifts to routine tasks). Another explanation of why high incomes fail to translate to happiness is related to the fact that as income rises, an individual's time use often does not shift toward activities associated with improved affect. Subjective well-being is connected to how people spend their time. The activities in which wealthier people spend relatively more of their time are associated with no greater happiness, on average, but with slightly higher tension and stress. Accordingly, the focusing illusion may be responsible for global judgments of life satisfaction being higher without increasing happiness. When asking people about their well-being, results differ when using a moment-to-moment measure (either collected in the present moment or by asking them to recall feelings during an episode the previous day) compared with global judgments of life satisfaction or overall happiness or a global report of yesterday's mood.

Despite the weak association between income and experienced happiness, most will work very hard to earn more money. The focusing illusion can lead to misallocations of time if one's objective is increased happiness, for example, accepting lengthy commutes (which are among the worst moments of the day) to sacrificing time spent socializing (which are among the best moments of the day).

Kahneman D, Krueger AB, Schkade D, Schwarz N, Stone AA. Would you be happier if you were richer? A focusing illusion. Science. 2006;312:1908-10.

Editor's Comment: *A 13-year-old boy is referred to a pediatric endocrinologist for an evaluation of short stature. "How are you doing?" the doctor asks. The context of the visit to the growth specialist and extra attention directed toward accurate height measurement makes it clear to the child and his accompanying parent that the doctor is really asking, "How are you doing being short?" At that moment, the child and parent will likely focus on events of height-related name-calling or incidents of being handed a child's menu at the restaurant. The child*

hesitates to respond (fairly typical of youth this age in such circumstances), so the mother replies, "He's very upset about his height." The growth chart reveals this young man meets criteria for idiopathic short stature. The physician may conclude he is a good candidate for treatment with growth hormone (GH) because he meets anthropometric criteria and is also suffering from experiences related to his diminutive size.

The analysis of Kahneman and colleagues suggests we would likely arrive at quite a different impression of the child's psychosocial and emotional adaptation if we were to assess these in a manner that does not bias attention toward a single factor: height. Studies which mask "height" as the variable of interest suggest that youths who are markedly shorter than average are, by and large, indistinguishable in their self-reports and in descriptions by their peers from those of average or tall stature with respect to their reputation, the number of reciprocated friendships, and their likeability.[1]

Setting aside these and related findings,[2] *there is a reasonable likelihood that this youngster would receive treatment based on complaints of psychosocial stressors and insistent parents. Should we anticipate that treatment will improve this youth's mood state? (Keep in mind that, by asking different questions, we would likely learn this teen's mood is better than our perfunctory evaluation suggests, and that height is far less salient in his life on a moment-to-moment basis than we are led to believe.) Or, based on this focusing illusion, might we predict that the experience of daily GH injections and regular visits to the pediatric endocrinologist for repeated height measurements and physical exams will increase the likelihood that the youth and parent focus on growth and height not only during the visit but also on a daily basis? If, as suggested elsewhere*[3] *by the same group of investigators that, "nothing that you focus on will make as much difference as you think," then taking children and their families down this road might be quite counter-productive. An alternative would be to embed a psychosocial component within the medical evaluation and shared decision-making with the family. Independent of whether a decision to initiate GH treatment is made, a psychosocial intervention to address on-going psychosocial stresses associated with short stature would likely result in improved daily function and increased patient happiness and parent satisfaction. Suggestions on how such an interdisciplinary model of care could be implemented have been described.*[4]

David E. Sandberg, PhD

References

1. Sandberg DE, Bukowski WM, et al. Pediatrics. 2004;114:744-50.
2. Ulph F, Betts P, Mulligan J, Fung CM, et al. Arch Dis Child. 2004;89:17-21.
3. Schkade DA., Kahneman D. Psychol Sci. 1998;9:340-6.
4. Colsman M, Sandberg DE. Psychological assessment. In: Kelnar CJH, Savage MO, Saenger P, Cowell CT, eds. Growth Disorders. 2nd ed. London: Hodder Arnold; in press.

Tolvaptan, A Selective Oral Vasopressin V_2-receptor Antagonist for Hyponatremia

Hyponatremia due to increased secretion of antidiuretic hormone (ADH) may be due to the syndrome of inappropriate secretion of ADH (SIADH) related to an insult to the central nervous secretion (or rarely in children—ectopic secretion of ADH), heart failure, or hepatic cirrhosis. Pathogenetically, it is the result of excessive and inappropriate reabsorption of free water in the renal collecting ducts in response to ADH signaling through the V_2 receptor (OMIM 300538, chromosome Xq28), a G-protein coupled receptor that stimulates adenylyl cyclase and generation of cyclic AMP. ADH is a cyclical 9 amino acid peptide derived from a larger parent protein that also contains within its structure neurophysin—a carrier of ADH—and a glycoprotein. Parenterally administered non-peptide antagonists to ADH have been developed to block the action of ADH in the renal collecting tubule by binding to the V_2 receptor and increasing the urinary excretion of free water (Figure 1).[1] In a randomized, double-blind, placebo-controlled, out-patient study in which fluid intake was not monitored, the investigators ascertained the efficacy and safety of the oral administration of one such agent, tolvaptan, in 171 adults (>18 years of age) with hyponatremia (120-134 mEq/L), 91 of whom had SIADH. Compared to placebo, tolvaptan rapidly and safely increased and maintained serum sodium concentrations into the low normal range over a 30-day interval of treatment (Figure 2). One week after discontinuation of tolvaptan, serum sodium levels declined to values seen in the group that received the placebo. As anticipated, tolvaptan increased urine output initially. The drug was well tolerated. The authors concluded that orally administered tolvaptan is a clinically effective V_2-receptor antagonist in adults with hyponatremia of diverse etiology.

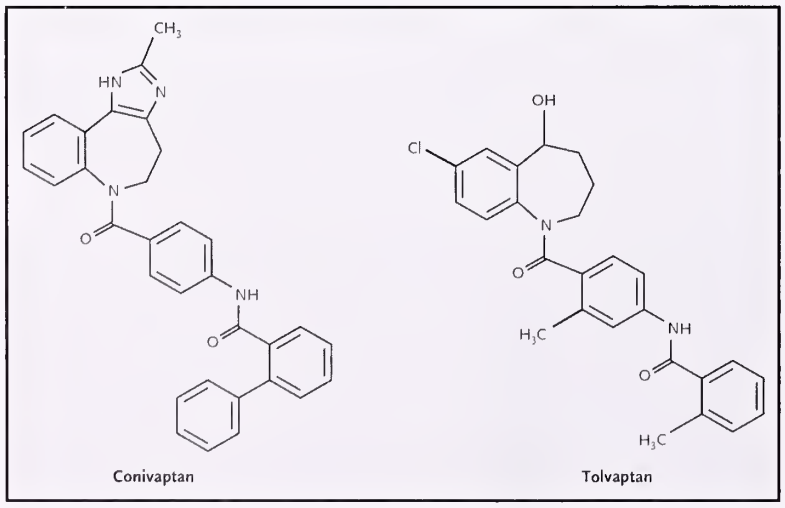

Figure 1. Structure of the Oral Vasopressin-Receptor Antagonists Conivaptan and Tolvaptan. Reprinted with permission from: Hays RM. N Engl J Med. 2006;355:2146-8. Copyright © MMS. 2006. All rights reserved.

Schrier RW, Gross P, Gheorghiade M, et al. Tolvaptan, a selective oral vasopressin V_2-receptor antagonist, for hyponatremia. N Engl J Med. 2006;355: 2099-112.

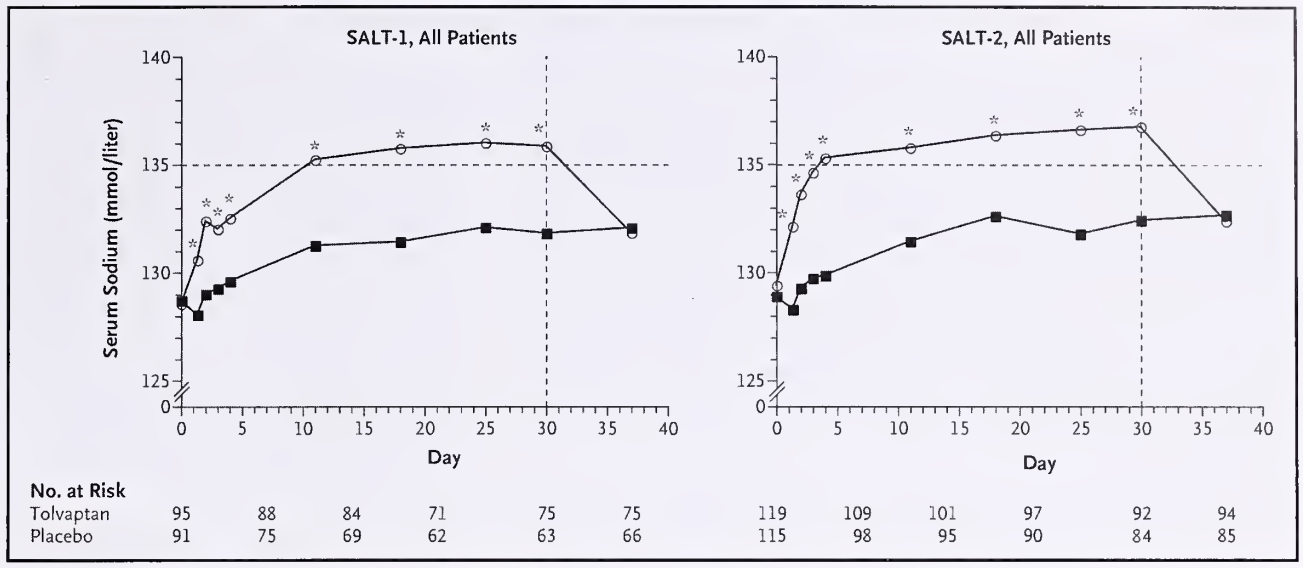

Figure 2. Mean serum sodium concentrations attained with tolvaptan (circles) and placebo (squares). Reprinted with permission from: Schrier RW, et al. N Engl J Med. 2006;355:2099-112. Copyright © MMS. 2006. All rights reserved.

Editor's Comment: *Management of SIADH in children is primarily accomplished by fluid restriction. In critical situations slow intravenous infusion of 3% saline may be considered in an amount calculated to increase the serum sodium concentration to values that ameliorate symptoms (approximately 125 mEq/L) while carefully monitoring urine output.*[2] *Very rapid increase in serum sodium concentrations may lead to central pontine myelinolysis. The non-peptide antagonists of the V_2 receptor have not been examined or approved for use in children as yet, but would appear to be promising therapeutic agents that have been termed "aquaretics." In addition to the renal V_2 receptor, there are V_{1a} and V_{1b} receptors that mediate the vasoconstrictive and adrenocorticotropin-releasing properties of ADH.*

Allen W. Root, MD

References

1. Hays RM. N Engl J Med. 2006;355:2146-8.
2. Robertson GL. Clinical disorders of the posterior pituitary. In: Pescovitz PH, Eugster EA, eds. Pediatric Endocrinology: Mechanisms, Manifestations, and Management. Philadelphia, PA: Lippincott Williams & Wilkins;2004:90-107.

Growth Hormone Treatment in Cystic Fibrosis

This multi-center study assessed auxological, respiratory, bone health, and quality of life variables in 61 pre-pubertal children with cystic fibrosis (CF) who were randomized to receive either growth hormone (GH [0.3 mg/kg/wk]) or no GH for one year. At the end of one year, there was cross-over and those who received GH stopped therapy and those not on treatment started GH therapy. Both groups were then followed for a second year. Significant improvements in gain in height velocity, weight, lean body mass, bone mineral content, quality of life, and hospitalization rates were demonstrated in the subjects treated with GH. Improvements were also maintained following discontinuation of GH.

Hardin DS, Adams-Huet B, Brown D, et al. Growth hormone treatment improves growth and clinical status in prepubertal children with cystic fibrosis: results of a multicenter randomized controlled trial. J Clin Endocrinol Metab. 2006:91:4925-9.

Editor's Comment: *Hardin is one of the few pediatric endocrinologists who is successfully addressing the problems of chronic severe childhood illness on growth, puberty, bone health, and quality of life. She and her co-authors are to be congratulated on this publication, which not only reports impressive positive results of GH therapy in children with CF, but can also be considered a notable achievement in terms of interdisciplinary collaboration. A defect in GH action is predictable from the effect of chronic infection and inflammation in children with CF. Insulin-like growth factor (IGF)-I levels have also been shown to be correlated with BMI and disease activity score, which as stated in this paper, may relate to long-term morbidity and mortality.*

It is pertinent to ask why convincing results such as these are apparently not changing clinical practice more rapidly. Poor interdisciplinary discussions and interchange must be responsible. Sub- or super-specialization within pediatrics may be considered synonymous with progress, but barriers can be constructed which make interspecialty and joint clinical management difficult. Pediatric endocrinologists are in a position to collaborate with many subspecialists, such as gastroenterologists, rheumatologists, hematologists, etc. Of course, stretched resources may make this difficult. However, as Hardin and her collaborators have demonstrated in this important study, the patient may have a great deal to gain from closer working relationships between colleagues in different pediatric disciplines. Well done!

Martin O. Savage, MD

MC2R Loss in Salt-losing Adrenal Hypoplasia

Familial glucocorticoid deficiency type I (FGD1) is a rare form of primary adrenal insufficiency resulting from mutations in the ACTH receptor (*MC2R*). These children typically have severe neonatal symptoms and signs of cortisol deficiency. The issue of mineralocorticoid deficiency is not well documented in this group and it is widely accepted that classic cases do not require mineralocorticoid replacement. However, hyponatremia has been observed in some patients, not related to hypocortisolism.

Lin et al considered the possibility of alterations in mineralocorticoid control since the *MC2R* is also expressed in the aldosterone producing zona glomerulosa in the adrenal gland. Twenty-two children diagnosed with salt-losing forms of primary adrenal hypoplasia (19 isolated cases, 3 familial) were investigated. All children were negative for the 2 mutations known to be involved in adrenal hypoplasia: DAX1 and SF1. All subjects were investigated for *MC2R* mutations, after amplifying the entire coding region (exon 2).

The *MC2R* mutations were found in 3 kindreds, involving 9 patients; age at presentation ranged from 1 day to 19 months. The initial symptoms were pigmentation, hypoglycemia, jaundice, and failure to thrive. The mutational changes in all 3 families represented disruptive loss-of-function in the G-protein coupled receptor, including the first reported homozygous frameshift mutations. In kindred 1, the patient was diagnosed at 3 months: electrolytes were normal, but aldosterone was low for age with elevated plasma rennin activity (PRA) that improved with prolonged fludrocortisone treatment. Two cases were later diagnosed in family 2. One presented with elevation of PRA, the other sibllng had low aldosterone and developed hyponatremia during a severe viral illness. Kindred 3 presented early symptoms. The first child required fludrocortisone because of early salt-losing syndrome. The 2 subsequent siblings were treated before overt sodium imbalance.

Salt-losing forms of adrenal insufficiency are generally clear and occur in well defined conditions. However, if biochemical findings are subtle, the exact biochemical nature of the condition can be difficult to assess. *MC2R* mutations should be considered in patients with apparent mild disturbances in rennin-sodium homeostasis. These children could be misdiagnosed for primary salt-losing adrenal hypoplasia. The genetic finding has important implications for treatment, counselling and long-term prognosis.

Lin L, Hindmarsh PC, Metherell LA, et al. Severe loss-of-function mutations in the adrenocorticotropin receptor (ACTHR, *MC2R*) can be found in patients diagnosed with salt-losing adrenal hypoplasia. J Clin Endocrinol. 2007;66:205-10.

Editor's Comment: *The authors described important clinical and pathophysiological issues in a rare but often confusing disorder. The diagnosis of isolated cortisol deficiency is suggested by the clinical presentation. Assessment of the mineralocorticoid function in the affected neonate may be difficult. If aldosterone deficiency is partial, as it appears to be in these cases, repeated coupled evaluation of plasma aldosterone and PRA is necessary. Furthermore, severe inactivation of the ACTH receptor may have an impact on the control of aldosterone secretion and suggests a risk of a salt-losing condition. Therefore, it is necessary to identify the genetic defect in these patients, assessing the most severe end of the spectrum of FGD1. These patients paradoxically may require fludrocortisone therapy at critical periods of severe illness. As the authors suggested, these mutations may be found in a significant proportion of children with primary adrenal insufficiency who sometimes have minimal signs of salt loss.*

In addition, these data confirm the supportive role for ACTH in mineralocorticoid synthesis and secretion, especially in a time of stress. It is possible that, as observed in pseudohypoaldosteronism type 1 due to mineralocorticoid receptor defect, the need for mineralocorticoid replacement weans off during childhood. This requires careful follow-up, as the risk of salt loss may persist at times of stress or potential volume depletion. An ongoing clinical vigilance is necessary.

Raphaël Rappaport, MD

Mutant IGF-1R as Cause for Familial Growth Failure

Inagaki et al identified a family in which 2 members had severe growth failure and a mutant type 1 insulin-like growth factor receptor (IGF-1R). A 13.6-year-old girl presented with growth failure (height z-score –5 SD, sitting height z-score –5.2 SD, and weight z-score –2.5 SD), Tanner stage 2 pubertal development, and delayed bone age (9.7 years). She had experienced prenatal growth failure (birth length –4.9 SD, birth weight –3.1 SD), triangular facies, and acromicria. The father's height was –2.2 SD, the mother's height was –5.7 SD, and they were nonconsanguineous. A maternal aunt's height was –5.7 SD and she seemed otherwise healthy. The patient's 2.5-year-old brother had a height of –1.2 SD. The patient had an elevated basal IGF-I level (404 ng/mL), normal growth hormone (GH) response to clonidine stimulation, and no increase in either her IGF-I level after 4 days of GH treatment or her height z-score after 6 of months GH treatment (0.07 mg/kg/day).

Inagaki et al then performed in vitro studies to ascertain the molecular mechanism of this family's

growth failure. Sequencing revealed substitution of the phylogenetically highly conserved arginine at position 481 to glutamate (R481Q) in the IGF-1R of both the patient and the maternal aunt. This arginine is in the N-terminal fibronectin type III domain, and situated near the first disulfide bond (Cys 514) between the 2 α-subunits. Either wild-type or R481Q IGF-1R was over-expressed in NIH-3T3 fibroblasts to conduct functional assays. R481Q IGF-1R altered neither surface expression nor ligand binding capacity. However, as demonstrated by Western blotting under reducing and non-reducing conditions, the mutant receptor had incomplete dimerization likely related to impairment of that first disulfide bond; the mutant, but not wild-type, IGF-1R showed monomeric forms of the β-subunit under non-reducing conditions. Further, compared to wild-type, R481Q IGF-1R had blunted IGF-I induction of IGF-1R autophosphorylation, p42/44MAPK phosphorylation, Akt phosphorylation, and cellular proliferation.

Thus, the authors concluded that R481Q disturbs the first disulfide bond of IGF-1R, thereby impairing its dimerization and ligand-stimulated conformational change that is required for signal transduction. This translated clinically into IGF-I resistance and growth failure.

Inagaki K, Tiulpakov A, Rubtsov P, et al. A familial insulin-like growth factor-I receptor mutant leads to short stature: clinical and biochemical characterization. J Clin Endocrinol Metab. 2007;92:1542-8.

Editor's Comment: *The authors astutely recognized the severe pre- and post-natal growth failure of their patient as indicative of reduced IGF-I activity; measurement of basal IGF-I concentration quickly ruled out IGF-I deficiency in favor of IGF-I resistance. The authors are to be commended on their detective work, which led to the discovery of a novel mechanism of IGF-I resistance that joins the short list of previously reported IGF-1R mutations.*

This illustrative case also highlights the importance of obtaining a good family history in the evaluation of a poorly growing child. Most often, we ascribe similar multigenerational height z-scores to familial short stature, which is considered a normal variant. However, when the growth failure is severe and affects a subset of relatives, as exemplified by this patient's family, an inherited growth defect should be considered. Another example would be autosomal dominant (type 2) isolated GH deficiency.[1,2] *Although the child may be short "like the parent," it is possible that they are sharing an underlying pathologic process.*

Adda Grimberg, MD

References

1. Binder G, Brown M, Parks JS. J Clin Endocrinol Metab. 1996;81:4047-50.
2. Salemi S, Yousefi S, Lochmatter D, et al. Endocrinology. 2007;148:45-53.

Ultimate Height of Growth Hormone Deficient Patients who Normalized Growth Hormone Secretion in Puberty

The objectives of the study by Zucchini et al were to establish the percentage and the characteristics of subjects diagnosed with isolated growth hormone deficiency (GHD) in childhood who normalized their GH secretion in puberty and discontinued treatment at that time. The final height attained by this group was compared with that of subjects with persistent GHD who continued on GH therapy after retesting. Sixty-nine subjects (40 males, 29 females) with a diagnosis of isolated GHD before puberty were evaluated by means of arginine and l-dopa testing and were reevaluated after at least 2 years of GH therapy and after the onset of puberty. If peak GH levels were >10 µg/L therapy was withdrawn.

At retesting, 44 subjects (63.7%; 24 males, 20 females) had a peak GH <10 µg/L. Patients with confirmed GHD were not different from subjects with normalized GH secretion regarding: height

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deficit at diagnosis, first year growth response to GH, age and height at onset of puberty, or height and insulin-like growth factor (IGF)-I at retesting. Males who continued treatment achieved an adult height of 165.1 ± 4.5 cm, while those who suspended therapy after retesting had an adult height of 164.0 ± 3.4 cm. Final height of females who continued treatment was 153.2 ± 4.1 cm, whereas those who suspended therapy after retesting were 152.9 ± 5.2 cm. Duration of therapy and GH levels at diagnosis and at retesting were found to be unrelated to achieved adult height or to height increments obtained during the period of observation.

Zucchini S, Pirazzoli P, Baronio F, et al. Effect on adult height of pubertal growth hormone retesting and withdrawal of therapy in patients with previously diagnosed growth hormone deficiency. J Clin Endocrinol Metab. 2006;91:4271-6.

First Editor's Comment: *A significant number of children diagnosed with GHD before entering puberty, particularly those with non-severe GHD not associated with multiple pituitary hormone deficiencies or with alterations of the pituitary anatomy, display normal GH secretion when retested after the completion of puberty. This discrepancy in GH testing before and after puberty could be the result of a transient deficiency which tends to normalize with the secretion of gonadal steroids or could be due to the unreliability of pharmacological tests when repeated over time.*

Zucchini et al suggested that GH retesting should take place at midpuberty when GH secretion rises and not after the attainment of final height and completion of puberty, thus avoiding unnecessary treatment of subjects who have normal GH secretion when retested at puberty. The final height attainted by subjects with normal GH secretion at retesting, who discontinued therapy, was similar to that of individuals with confirmed GHD who continued treatment. Therefore, the withdrawal of GH therapy after retesting did not lead to a reduction in ultimate height. There were no clinical or laboratory parameters that allowed for the differentiation of patients with or without persistent GHD after puberty. In subjects with non-severe GHD it seems advisable to retest GH secretion during puberty and to discontinue treatment in those individuals that are no longer deficient, thus avoiding unnecessary treatment during and beyond puberty in these subjects.

Roberto Lanes, MD

Second Editor's Comment: *Several years ago we described the recovery of patients with isolated suboptimal GH secretion after a short trial of GH releasing hormone (GHRH).[1] Why wait until puberty?*

Fima Lifshitz, MD

Reference

1. Lifshitz F, Lanes R, Pugliese M, et al. J Clin Endo Metal. 1992;75:1255-60.

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In This Issue
Reviews & Comments

DISORDERS OF SEX DEVELOPMENT: MAKING AMBIGUITY LESS AMBIGUOUS

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INTRODUCTION

The recent consensus conference[1,2] on intersex, subsequently referred to as disorder(s) of sex development (DSD), made several important in-roads towards the establishment of an internationally endorsed intersex management guideline; nevertheless, some very fundamental questions remain. Some of the most contentious topics simply were not considered for consensus because of a lack of outcome data on which to anchor objective assessment. Other topics, such as how to make management decisions in the absence of objective data, were not approached.

The recent controversy in the medical management of DSD has arisen primarily due to the complaints of adult DSD patients about the lack of full disclosure, absence of participation in treatment decisions, minimal privacy about their condition, and the consequences of genital surgery. There has also been criticism centered on the use of the "optimal gender" concept for gender assignment, an approach that viewed gender as a largely social construct whose predominant influence was sex of

From The Editor's Desk

Dear Colleague:

The publication of this issue was made possible through an unrestricted medical education grant from Pfizer, Inc. We also received support from Pediatric Sunshine Academics, Inc. and from many individual donations from our loyal readers. This allowed us to close the year with a big bang; the printed issue of Volume 23, Number 3 of GGH was expanded to 28 pages. On behalf of the editorial board and all of our readers I want to express my deep and most sincere appreciation to the sponsors who made possible the publication of this highly regarded journal.

The lead article of this issue was written by Drs. Christopher Houk and Peter Lee—it deals with a subject of great current interest, "Disorders of Sexual Development: Making Ambiguity Less Ambiguous." In addition there are 22 reviews of peer-reviewed papers published in the recent literature, each one of them with erudite editorial comments. Additionally, we will be offering the capability of obtaining CME credits for reading GGH. CMEs will be available online at our website www.GGHjournal.com—click CME.

We will continue to search for the support that is needed to produce a high quality medical education journal for pediatric endocrinologists and for all those interested in the field. The long-term support through a single sponsor that GGH enjoyed in the past is currently not available, thus we will hope to elicit partial funding from multiple sources to be able to provide you with GGH throughout 2008 and thereafter.

Should you wish to contribute to the journal's success please make a special year-end tax-deductible contribution either on line (www.GGHjournal.com or www.PedsAcademics.org) and click make a donation, or send a check to Pediatric Sunshine Academics, Inc. 1040 Alston Road, Santa Barbara, CA 93108.

Thank you for your interest in and support of GGH.

Fima Lifshitz, MD
Editor-in-Chief

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rearing.[3] Because postnatal factors were felt to carry great weight for the determination of gender identity, and because DSD management was felt to be complex and confusing, parents were given little information and little input in the decision-making process. In this model, sex assignment was based on potential for fertility, penile-vaginal sexual intercourse, and near-normal appearing genitalia—factors felt to be important for adult quality of life. This paradigm held that when sex assignment was followed by genital surgery, a gender identity consistent with the gender assignment would ensue. This was felt to be true in spite of the knowledge that 46,XX individuals exposed to androgens in utero were more likely to exhibit more male-type gender role behavior,[4,5] and, although not well documented, were felt to be more likely to be sexually oriented towards other females.[6,7]

Over recent decades, it has become obvious that some of the core assumptions about the impact of sex of rearing were incorrect. This has been especially true for 46,XY DSD patients[8,9] although issues in virilized 46,XX patients have also emerged.[10,11] While inconsistent gender roles and homosexual orientation are not as imposing issues in current society as they were in the past, gender identity opposite to that assigned during infancy suggests a need for better guidelines. While the relative roles played by the various biological and social determinants of gender remain an enigma, there is accumulating evidence that prenatal influences, particularly those related to androgen exposure during fetal life may influence gender identity.[9,12,13,14] Furthermore, the position that quality of life could be predicted by the extent to which a DSD individual had normal appearing genitalia or ability to participate in traditional sexual activity is too limited in scope to be helpful to an individual patient. In some cases the attempt to create anatomically 'correct' genitals resulted in reduced genital sensitivity and erotic function such that one of the primary goals of surgery—namely satisfying sexual activity—was sacrificed.[15,16] Given the degree of alienation reported by parents of DSD children, it was felt that a new treatment paradigm should be developed—one that allows a more informed, evidence-based approach to the DSD patient.

REVIEW OF CONSENSUS STATEMENT

The consensus conference (comprised of members of the Lawson Wilkins Pediatric Endocrine Society [LWPES] and the European Society for Paediatric Endocrinology [ESPE]) formed a position when they had reached a collective opinion that there was adequate outcome data to support a position statement and when there was sufficient agreement on the topic. One example of the groups' opinion was seen in the position that genital surgery should be undertaken for the severely ambiguous infant when parents request it, despite the absence of outcome data to show that this approach is superior to its alternative. Although it was agreed that the impact of prenatal androgen exposure is important to gender outcomes, the group was uncomfortable recommending that treatment be based on an estimate of intrauterine androgen exposure. Another example of this is seen in the groups' reluctance to recommend that a male sex of rearing be considered in a fully virilized (and hence highly androgen exposed) 46,XX infant with congenital adrenal hyperplasia (CAH). A consensus could not be reached for 2 of the more controversial issues: (1) establishing clear indications for sex of rearing for severe genital ambiguity and (2) establishing complete guidelines on when genital surgery should be deferred. However, it was agreed that surgery should not be done in mildly virilized genetic females (Prader stage 1 or 2).

SEX ASSIGNMENT

Guidelines for gender assignment were addressed only for those DSD patients with substantial outcome data. A review of outcome studies show that: (1) >90% of virilized 46,XX CAH patients identify as females; (2) 100% of 46,XY complete androgen insensitivity syndrome (cAIS) patients identify as female; (3) 100% of 46,XY 5α-reductase deficient patients assigned male at birth identify as male, while more than half of those assigned female who virilized at puberty live as males;[17,18] and (4) approximately 50% of 46,XY 17β-hydroxysteroid dehydrogenase (17βHSD) deficient patients assigned female ultimately switch to the male gender. Accordingly, it was recommended that all 46,XX CAH and 46,XY cAIS patients be assigned female. It is important to note that this recommendation was made in spite of a small number of documented cases of initially undiagnosed extremely virilized 46,XX CAH patients assigned male at birth, who maintained the male gender in adulthood and developed a sexual orientation towards females.[19] A male sex of rearing was also recommended for 46,XY patients with 5α-reductase deficiency and 17βHSD deficiency (Table 1). The consensus found outcomes for ovotesticular DSD to be so variable that sex assignment recommendations should be based on fertility potential, assuming consistent genitalia.

Table 1. Current Diagnosis-based Recommendations for Sex of Rearing

1. Complete androgen insensitivity syndrome (46,XY cAIS) - female
2. Partial androgen insensitivity syndrome (46,XY pAIS) - dependent upon judgment of degree of masculinization and parental input
3. Congenital virilizing adrenal hyperplasia (46,XX CAH) - female, realizing that there are anecdotal reports, but not verified documentation, of those with essentially male external genitalia raised satisfactorily as male
4. 5α-reductase deficiency - strongly consider male assignment
5. 17β-hydroxysteroid dehydrogenase-3 deficiency - strongly consider male assignment
6. Cloacal extrophy - conflicting outcome data, reports from the US show high rates of self-reassignment to male
7. Ovotesticular DSD - consider external genital development and fertility potential; given outcome uncertainties potential for fertility (assuming consistent genitalia) is a major factor

The consensus did not find the outcome studies sufficiently clear to permit a sound recommendation for sex assignment in 46,XY patients with cloacal extrophy or partial AIS (pAIS). The diagnosis of pAIS is particularly problematic, because the criteria used to establish it are vague and the outcomes seen are so variable, resulting in etiologic heterogeneity within this group of DSD patients. Given these uncertainties in all DSD patients, physical

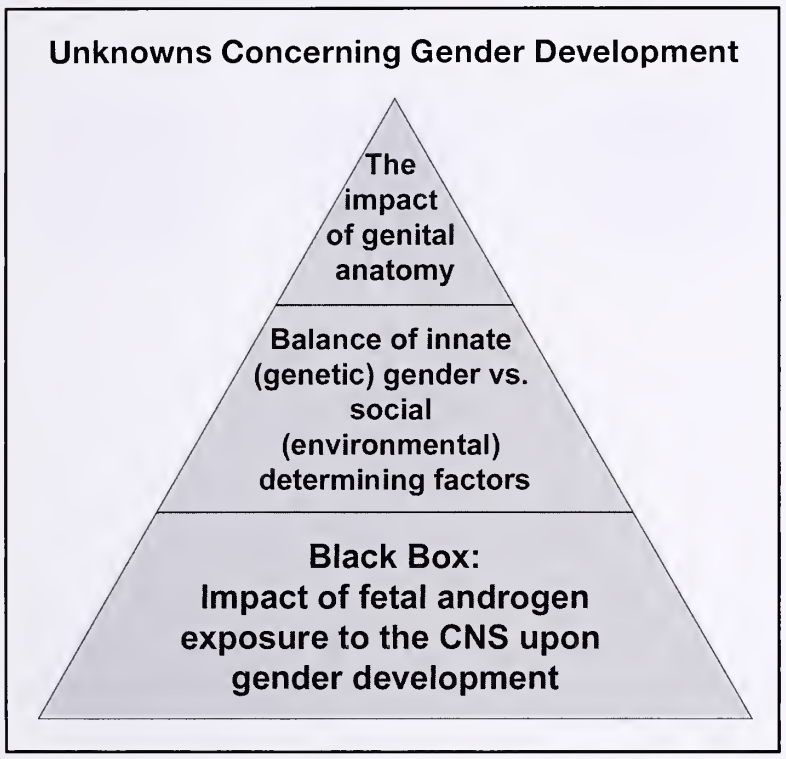

Figure 1. It is doubtful whether the unknowns represented in this figure will ever become certain enough to provide precise guidance for sex assignment. All aspects of psychosexual differentiation may not be equally affected by prenatal androgen exposure.

findings alone cannot be used to justify gender assignment. In point, it was felt that the degree of fetal virilization cannot be used as a surrogate of fetal CNS androgen exposure (Figure 1). Accordingly, the use of genital anatomy to guide gender assignment or predict future gender identity should be deemphasized for determining sex of rearing.

It is the authors' opinion that in these cases the DSD team and the parents must decide whether the genitalia (in some cases, only after androgen stimulation), considered alongside other factors (karyotype, social issues, gonadal function, etc) would permit successful male sex of rearing. The basis of gender assignment and the relative weight of the various interrelated factors are shown in Figure 2. Although other factors should not be underestimated, family support is of capital importance in determining a successful sex assignment (Figure 3). In cases of undervirilized 46,XY patients, initial sex assignment should be approached cautiously given the outcomes seen in this group of DSD patients. In particular, the prospect for 46,XY DSD patients assigned female at birth who then later developed disabling gender dysphoria should be discussed with the family. This is especially true of an idiopathic undervirilized 46,XY patient with evidence of testicular function. In patients with micropenis, it was

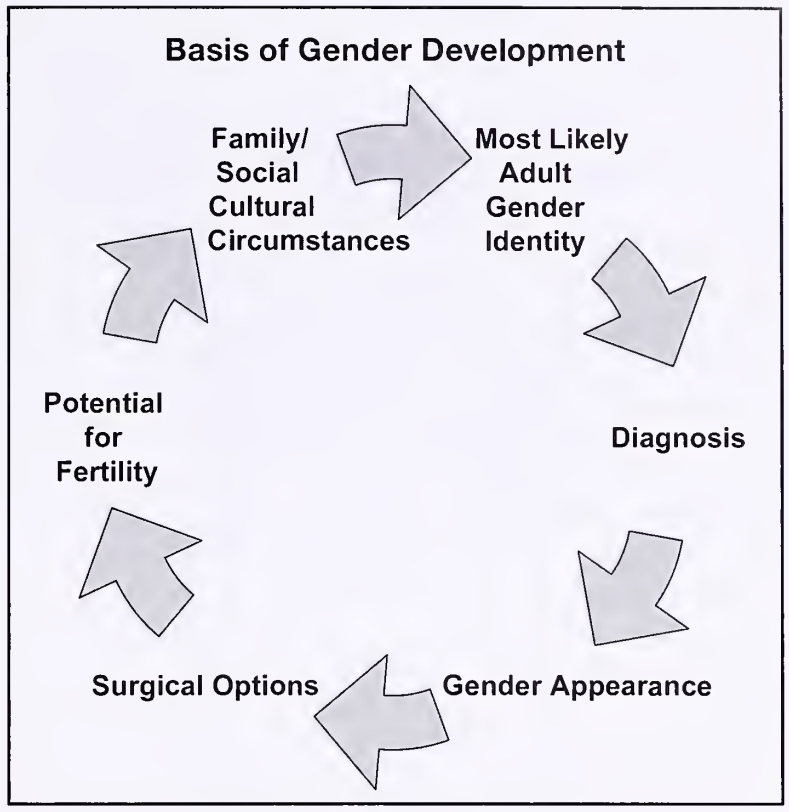

Figure 2. The relative weight of the sex assignment inter-related factors differ in each situation, largely by unknown and non-predictable influences. The magnitude of the impact of each factor upon the others is also variable over time.

the consensus that all should be raised male, based on considerations that include the lack of need for surgery, fertility and adult male gender identity.

In terms of gender re-assignment, the consensus strongly felt that all gender re-assignment—that is, assignment after infancy—should only be undertaken after the gender identity is well established and should be patient initiated. Neither homosexuality nor cross-gender behaviors can be used as support that a gender assignment has failed. Decisions about the need for or utility of gender re-assignment should not be influenced by gender role or sexual orientation and should be solely based on the presence of a durable gender identity.

SURGICAL ISSUES

The consensus found that the anecdotal reports of DSD individuals dissatisfied with previous genital surgeries was inadequate and insufficiently compelling to recommend that a moratorium be placed on all genital surgeries. The consensus, based upon the recommendations of the surgical subgroup, agreed that the primary goal of genital surgery was to improve functional rather than cosmetic outcome.[1] This represents a shift towards a more conservative treatment approach, but is far short of suggesting that all genital surgery be deferred. For the severely virilized 46,XX infant with CAH—the most frequent cause of severe genital ambiguity—early genital surgery is recommended. For 46,XX infants with mild/moderate clitoromegaly, it was advised that genital surgery be deferred until patients can participate in

the decision. Given the absence of outcome data showing that it is harmful, it was felt that parental rights and responsibilities as surrogate decision-makers should be respected in cases where informed parents continue to request early genital surgery for their DSD child. This situation further highlights the need for data-driven recommendations.

In addition, outcome data were used to stratify gonadal malignancy risk for specific DSD etiologies and evidence-based recommendations were made on the need for and timing of gonadectomy.[1]

CURRENT STANDARD

The consensus highlighted several standards of care recommendations: (1) gender assignment for all; (2) avoidance of assignment before expert evaluation; (3) open communication; (4) a multidisciplinary team approach; and (5) confidentiality and attention to patient and family concerns.[1] It was emphasized that patient and family concerns be carefully considered and that adolescent patients should be offered the strictest confidence. This must take into consideration the fact that parents may not be ready to agree with full disclosure to their children. Hence, while respecting this, adolescent patients should be given opportunities to ask questions and discuss their condition confidentially, without their parents being present. The basis of gender assignment (Figure 2 and Table 2) should include: (1) the most likely adult gender identity; (2) diagnosis; (3) genital appearance; (4) surgical options; (5) potential for fertility; and (6) family/social/cultural circumstance. Data are not available to predict outcome in the majority of DSD patients, therefore the consensus felt that decisions based on psychosocial factors, such as parental wishes, should guide management decisions in cases with uncertain outcomes. This approach recognizes the powerful influence of parental input on outcomes (Figure 3).

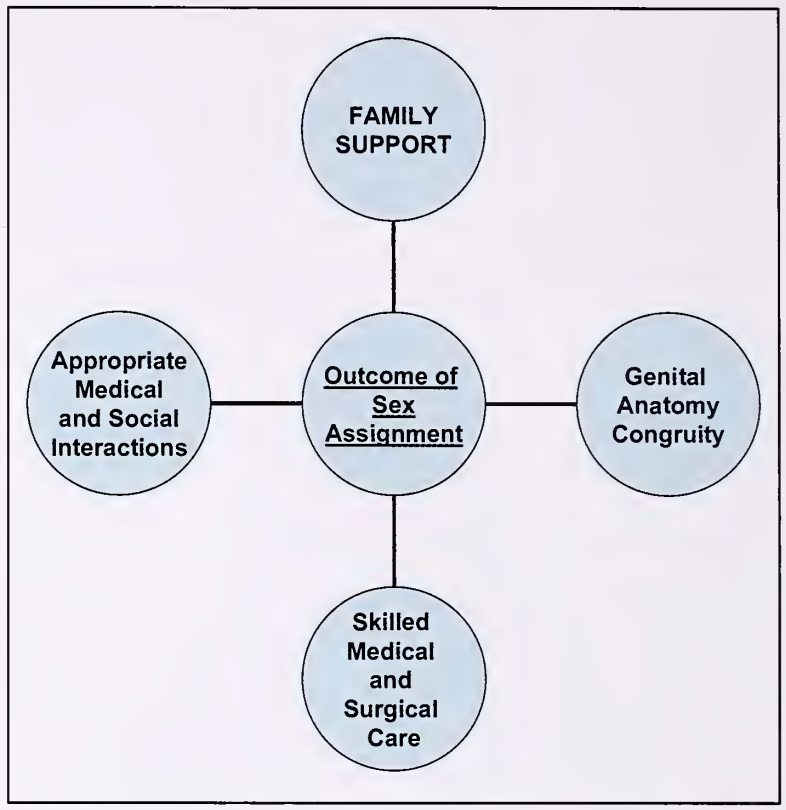

Figure 3. The primary factor that appears to promote a well-adjusted outcome after sex assignment is family support. Further, the importance of the family's role in assuring supporting medical and social contacts and exchanges, demanding skilled medical and surgical care, and interpreting genital anatomy as appropriate should not be underestimated.

Table 2. Recommendations for Gender Assignment: Factors to Consider

1. Most likely adult gender identity based on impression of fetal androgen exposure, parents' expectations and expected impact of sexual differentiation
2. Diagnosis, if specific diagnosis available and outcome data available
3. Genital appearance (as primarily male, female or intermediate)
4. Genital surgical options (potential for functional, sensitive genitalia)
5. Potential for fertility, considering assisted fertility techniques including intracytoplasmic sperm injection (ICSI) and donor ova
6. Social and cultural pressures
7. Family dynamics and social circumstances including parents' desires, expectations, malleability, and reactions to genital ambiguity
8. Depending on the degree of unpredictability of outcome, deference given to psychosocial factors when outcome is unpredictable

Medical decisions in the DSD patient are usually made in what has been referred to as Category III level of evidence (Category III: Opinions of respected authorities, based on clinical experience, descriptive studies and case reports, or reports of expert committees[20]). Thus, there are major limitations to making recommendations for DSD patients for most issues, particularly those most controversial topics of sex assignment and genital surgery, since the available evidence is considered to be Category III, which is the least compelling.

UNRESOLVED ISSUES

The effects of the care given to DSD patients are still not well understood, thus it has been difficult to determine which of the traditional and which of the more contemporary treatment approaches provide the optimal chance for a successful outcome. Despite the advances in our understanding of human sexual development, the sheer number of possible etiologies, the high degree of phenotypic heterogeneity and the overlay of psychological/sexual/social/cultural pressures in DSD patients make it difficult to develop comprehensive guidelines for approaching these issues. This difficulty is compounded by the paucity of outcome data for DSD patients. These factors notwithstanding, it remains important to sort out which questions will be answerable with new information versus those (such as which gender assignment should be proposed in all cases) questions which may never be answerable.

DECISION MAKING

The consensus conference recognized the role of various entities in decision-making for the DSD child including: the parent, the child, and the medical system (Figure 4). However a practical application of these roles and ways to resolve potential conflicts between decision makers were not addressed. The role of other parties, such as advocacy groups, in decision-making was also not addressed. This pertains to those who have an interest in DSD issues based on ethical, human rights, and legal grounds. However, the potential role that support groups can play to facilitate better understanding of DSD patients or improving their quality of life was recognized. Health care professionals were encouraged to offer participation in support groups, although a practical way to broadly implement this was not addressed.

NEED FOR OUTCOME DATA

The need for outcome data from a large cohort of randomly selected DSD patients is obvious (Table 3). However, the 1999 North American Task Force on Intersexuality, which was established to address this problem, was unsuccessful for several reasons. These included HIPPA restraints, investigators' hesitation to pool data, and patients' reluctance to participate. Hence, the risk of non-representative sampling, the rarity of the disorders, the variability with which patients are managed, and the lack of a well-capitalized central data-collection facility may be factors that will continue to preclude an appropriately designed study. Ideally the collection of such data would involve a centralized collection point and be similar to other NIH/federally/foundation funded projects, such as those seen in other pediatric subspecialties relating to malignancies, hemophilia, or cystic fibrosis. It is tempting to think that support groups could serve as a conduit to help capture the additional data needed to answer some of these questions.

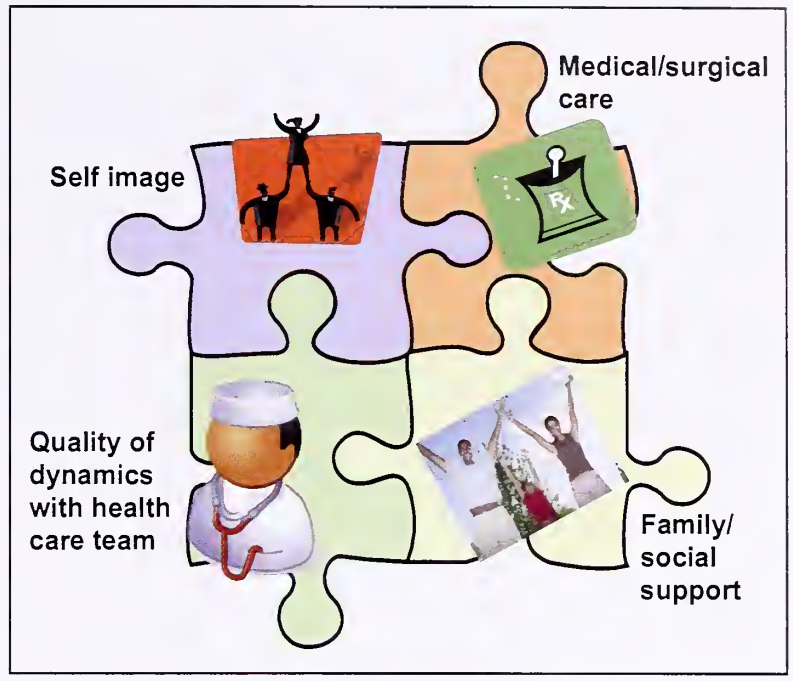

Figure 4. The puzzle is far from complete. The importance of medical care, support by adults including parents, understanding health care workers, and self-perceptions are obviously key.

Table 3. Challenges for Future Management and Studies of DSD

1. How to manage a complex medical condition without outcome data?
   a. Expert opinion
   b. Expert consensus
2. How to study DSD in children to assess newer therapies and approaches?
   a. Case reports (including data from advocacy groups)
   b. Case series (theoretical concepts extrapolated from existing data)
   c. Case-control studies
   d. Cohort studies
   e. Randomized controlled double-blind studies
   f. Systematic review/meta-analysis
3. What is the role of early genital surgery?
   a. Patients psychological and psychosexual adjustment
   b. Normalcy of appearance of genitalia
   c. Requirement for subsequent surgery, particularly vaginoplasty
   d. Genital sensitivity related to adult sexual responsiveness
4. How to best engage support groups?
   a. Utilize availability at time of presentations and crises
   b. Collaborate in general and specific educational efforts
   c. Maintain congenial interactions
5. How to meet the need for psychologists?
   a. Enhance currently skilled psychologist availability
   b. Advocate for support via Health and Behavior CPT codes for psychologists involved in counseling of DSD patients and their families, thus providing a mechanism for which counseling can be reimbursed
   c. Support the education of psychologists interested in the psychosocial and psychosexual factors among DSD individuals

BARRIER TO BETTER STUDIES

Clear barriers to the study of children with DSD remain. In addition to the relatively poor funding designated to pediatric research, there are inherent issues of recognizing and protecting the rights of children. Furthermore, study of children with DSD presents a unique set of delicate problems given that some of the most important questions revolve around gender identity, genitalia, and sexual function. While the popular literature and the press attempt to be realistic on issues of gender and sexuality, there continues to be a general lack of knowledge and hence a lack of understanding of issues facing the DSD patient. Thus, it would be difficult if not impossible to design, receive approval, and conduct any study tracking the development of human sexuality, particularly the evolution of sexual orientation and sexual function during childhood. It is clear that without answers to these developmental questions, it is much more difficult to anticipate outcomes for most DSD patients with any degree of certainty. It appears, as in most other chronic illness in children, that a good quality of life is largely dependent on a strong family support system (Figure 3). The plan for the care of DSD patients must accommodate cultural and religious sensitivities and incorporate realistic issues regarding DSD patients, such as childhood gender development and sexuality.

Currently, this is the context in which research and patient care concerning DSD must operate. A less contentious approach would be to design a case-control study comparing matched DSD patients with good outcomes to those with less than good outcomes to determine what, if any, management, family, or environmental factors are associated with outcomes.[21,22]

UNRESOLVED SURGICAL ISSUES

Curiously, the logic used to attack the traditional management paradigms has become one of the greatest hurdles to moving forward; that is, how does one develop a management model in the absence of clear outcome data for the disease/condition in question? The Columbia Court opinion[23] stated that children must be viewed as individuals with dignity and rights who are unable to give consent. This opinion states that while it is unclear whether or not genital surgery is necessary and urgent, it is also unclear whether the alternative of delaying surgery would work in society and would force a type of social experimentation. Given our degree of ignorance in DSD, the Columbia court citation declared that in cases where medical issues were unclear the ultimate decision-making rests with the parents—the "pro dubio in familia" rule—seems particularly wise and relevant.

Foremost in the discussion of modern genital surgery is the need for outcome data on genital sensitivity and function in adulthood following the use of modern, nerve-sparing genital surgery.[24] It is anticipated that function will be greatly improved over previous poor outcomes,[25] but the degree of improvement is at this point only theoretical. Unfortunately, confirmation of this improvement will not be possible for years. One problem with postponing genital surgery until the DSD child is capable of exercising a decision is the absence of a clear definition of the age and stage at which children can be expected to rationally make this decision.

PSYCHOLOGIST DEFICIT

The LWPES ad hoc Intersex Committee devoted many hours to the completion of the consensus conference to address the continuing need for experienced psychologists who could be part of care teams for DSD patients. However, there has been little progress towards meeting this need in our health care system. Most psychologists receive little training or experience with approaching a gender disordered or DSD patient. The net result has been that most psychologists remain uncomfortable with these patients. There are medical centers which are now supporting obesity centers, using a multidisciplinary approach, in an attempt to demonstrate that a preventative and multi-specialty approach will lead to healthier individuals and ultimately save health care dollars. Perhaps a similar attempt at one or more large medical centers with a functioning DSD team could develop a similar model.

SCIENTIFIC DEFICIT

The rapid advance of genetic knowledge has resulted in an expansion in diagnostic categories of the DSD patient. This will likely be helpful if it leads to better understanding of the biologic reproductive function in specific DSD types. Little progress has been gained concerning the age-old dilemma of genetic vs. environmental relative influence (nature vs. nurture). The need for funding agencies to support studies that serve to better understand genetic and endocrine factors of physical and psychological sexual differentiation is clear. Such funding should support the total purview impacting DSD issues; basic molecular and genetic research, differentiation using animal models, surgical techniques, physiological, and psychological, including psychosexual domains.

CONCLUSION

It is important that the caretakers of DSD patients avoid the allure of adopting untested management approaches out of frustration. Regardless of the intent, this sort of approach risks making the same mistake for which the traditional management model stands accused—that is, making medical decisions without a clear understanding of the disorder or most likely outcome in the absence of intervention. An example of this type of thinking would be to advise parents to forego all genital surgeries without providing them a basis on which to frame their own decisions. In this example, one type of prescriptive authority—that is, performing genital surgery on all DSD infants—is exchanged for another—that is, no DSD infant receives genital surgery. Any recommendations must be based either upon clear scientific data or on a synthesis of the effects of the complex interplay between biological, social, and psychological inputs. How parents are expected to make a truly informed decision about sex assignment is unclear. Based on anecdotal experience, it seems that parents often make their decisions in a way that is similar to those championed by the optimal gender approach. A concern about this is that most

Table 4. Summary: State of Management of DSD

1. Diagnosis and management of DSD continues to be a confusing topic.
2. All DSD conditions that present major challenges concerning sex of rearing (gender assignment) issues are rare and have received limited scientific study.
3. As with other congenital or chronic conditions, associated stress of the condition can be greatly alleviated and adjustment enhanced by skilled psychological counseling.
4. In spite of current "enlightened generations", most people remain uncomfortable discussing human sexuality when it pertains to themselves or their children.
5. There are minimal outcome data to support existing management of DSD conditions.
6. Yet, to do nothing is a breech of standard of care.

new parents focus upon the issues at infancy and find it difficult to project their thinking in terms of optimal adult outcome. For example, they often chose surgery out of a desire to create normal appearing genitalia even when it is clear that this intervention might compromise genital sensitivity.

Overall, much work and collaboration is needed to address the multiple issues of DSD management. Most DSD infants are not born in close enough proximity to centers with experienced DSD care teams to allow this team aspect of management to be helpful for the majority of affected children. In summary (Table 4), the management of DSD is inherently confusing (Figure 4). It has become clear that outcomes in DSD patients seem to be best characterized with a multi-factorial disease model that presupposes the simultaneous co-alignment of genetic, physical, and environmental risks for disease expression. We have much to learn from DSD patients and it is our hope that this article will help to encourage a genuine discussion of the issues facing these rarely encountered and poorly understood patients.

References

1. Lee PA, Houk CP, Ahmed SF, Hughes IA and the International Consensus Conference on Intersex Working Group. Pediatrics. 2006;118:488-500.
2. Houk CP, Hughes IA, Ahmed SF, Lee PA on behalf of the LWPES/ESPE Working Group (2006). Pediatrics. 2006;118:753-7.
3. Money J, Hampson J, Hampson J. Bull Johns Hopkins Hosp. 1955;97:284-300.
4. Meyer-Bahlburg HF, Dolezal C, Baker SW, et al. Arch Sex Behav. 2004;33:97-104.
5. Berenbaum SA, Bailey JM. J Clin Endocrinol Metab. 2003;88:1102-6.
6. Zucker KJ, Bradley SJ, Oliver G, et al. Horm Behav. 1996;30:300-14.
7. Meyer-Bahlburg HF. Endocrinol Metab Clin North Am. 2001;30:155-71.
8. Imperato-McGinley J, Miller M, Wilson JD, et al. J Clin Endocrinol (Oxf). 1991;34:293-8.
9. Cohen-Kettenis PT. Arch Sex Behav. 2005;34:399-410.
10. Meyer-Bahlburg HF, Dolezal C, Baker SW, et al. Arch Sex Behav. 2006;35:667-84.
11. Meyer-Bahlburg HF, Gruen RS, New MI, et al. Horm Behav. 1996;30:319-32.
12. Berenbaum SA, Korman Bryk K, Duck SC, et al. J Pediatr. 2004;144:741-56.
13. Hines M, Brook C, Conway GS. J Sex Res. 2004;41:75-81.
14. Migeon CJ, Wisniewski AB, Gearhart JP, et al. Pediatrics. 2002;31:110:e31.
15. Minto CL, Liao LM, Woodhouse CR, et al. Lancet. 2003;361: 1252-7.
16. Crouch N S, Minto CL, Laio LM, et al. BJU Int. 2004;93:135-8.
17. Mendonca BB, Inacio M, Costa EMF, et al. Endocrinologist. 2003;13:201-4.
18. Cohen-Kettenis PT. Arch Sex Behav. 2005;34:399-410.
19. Money J, Dalert J. J Homosexuality. 1976;1:357-71.
20. U.S. Preventive Services Task Force. Guide to Clinical Preventive Services 2nd ed. Baltimore, MD: Williams & Wilkens. 1996.
21. Money J. Int J of Family Psychiat. 1984;50:317-39.
22. Money J. Int J of Family Psychiat. 1984;50:341-73.
23. Columbia Decision. Intersex Society of North America (http://www.isna.org/columbia).
24. Baskin LS. J Pediatr Endocrinol Metab. 2004;17:581-7.
25. Creighton SM. BJU Int. 2004;93(S3):44-6.

Editor's Comment: *The topic of disorders of sex development is of high scientific and lay public interest. Among pediatric endocrinologists the interest in this topic has long been preeminent. Indeed, one of the most frequently read articles among the subscribers of GGH who have accessed the archives of the journal during the past 4 years is the lead paper by Sheri A. Berenbaum, PhD, entitled "Management of Children with Intersex Conditions: Psychological and Methodological Perspectives."*[1]

Among the highlights that stand out of the more recent papers dealing with this subject is the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology publication of their "Consensus Statement for the Management of Intersex Disorders."[2] *It is also worth pointing out that at the latest meeting of the LWPES (Toronto, Ontario, May 5, 2007) the State-of-the-Art Lawson Wilkins Lecture was "Ethical and Historical Considerations in Treating Children with Disorders of Sex Development and Idiopathic Short Stature" presented by Alice Dreger, PhD (Fienberg School of Medicine, Northwestern University, Chicago, Illinois). Additional publications that have attempted to bridge the scientific and the public understanding of the issues involved with individuals with disorders of sex development are: a recent book, "Ethics and Intersex" edited by Sharon Systma*[3] *and reviewed by Ieuan Hughes*[4] *and the article "What if It's (Sort of) a Boy and (Sort of) a Girl?"*[5] *The lead paper in this issue of GGH by Drs. Houk and Lee brings forth further clarifications of the intricacies of the management of these patients and the state-of-the-art concepts in this topic to make ambiguity less ambiguous. The ethical and legal implications of genetic testing in androgen insensitivity syndrome are also reviewed in this issue of GGH.*[6]

Fima Lifshitz, MD

References

1. Berenbaum SA. Growth Genet Horm. 2003;19:1-6.
2. Lee PA, Houk CP, Ahmed SF, Hughes IA. Pediatrics. 2006;118:e488-500.
3. Stysma S, ed. Ethics and Intersex (International Library of Ethics, Law, and the New Medicine. Vol. 29). Dordrecht, The Netherlands: Springer; 2006.
4. Hughes I. New Engl J Med. 2007; 356:2434-5.
5. Weil E. What if It's (Sort of) a Boy and (Sort of) a Girl? The New York Times Magazine. September 24, 2006.
6. Growth Genet Horm. 2007;23:40-41.

REVIEWS & COMMENTS FROM THE LITERATURE

Androgen Insensitivity Syndrome—Ethical and Legal Implications of Genetic Testing

Berg and colleagues presented a case of a 2-month-old full-term infant with an inguinal hernia. The external genitalia were unambiguously female; however, bilateral hernias with solid structures having internal blood flow but no follicles or Fallopian structures were detected. Hernia repair revealed seminiferous tubules with germ cell hyperplasia, no vas deferens, and presence of round ligament tissue. The karyotype was 46,XY and MRI of the pelvis revealed absence of the uterus, ovaries, and a blind vaginal pouch. The diagnosis of androgen insensitivity syndrome (AIS) was confirmed by identification of a novel homozygous nonsense mutation predicted to negatively impact androgen receptor (AR) gene function. The authors then provided an informative discussion of the syndrome and its clinical management.

In the context of counseling this particular family about the heritability of AIS, testing the proband's sisters was recommended. The patient's 22-month-old sister was diagnosed with AIS; and the 9-year-old maternal half-sister had a 46,XX karyotype. A four-generation pedigree detected a significant bias toward female offspring in previous generations; 10 of the 11 individuals in the great-great-grandmother's generation were female, 5 of whom were infertile and some of whom were known to have absent ovaries, uterus, or both. There were also women in more recent generations. The authors assumed many of these women were at risk for being previously undiagnosed 46,XY females or 46,XX heterozygous carriers of the familial AR mutation who could have affected children with future pregnancies. Due to the possible health risks associated with AIS (an increased risk of testicular neoplasms, which is reportedly greatest after puberty but can occur even in the elderly, and increased risk for osteopenia), provision of genetic testing for other at-risk family members could be considered an ethical responsibility of the health care team.

The ethical aspects of diagnostic disclosure elucidated were: (1) the history of withholding information from patients with disorders of sex development (DSD) based on the assumption that physicians were better able to determine what was in the patient's best interest; (2) the principle of informed consent asserts an ethical imperative to disclose such a diagnosis to the patient; in the case of minors, participation in decision-making is guided by the concept of "assent" commensurate with developmental capacity; and (3) the extent to which a physician has the dual responsibility to maintain confidentiality and to inform other members of the family that they may be at risk for being affected by a condition or for transmitting it to their offspring. The best resolution to the latter issue is to request that the parents of the affected child disclose their child's condition to other members of the family and ask those members to contact a physician. The authors advised, in the case of refusal to disclose information to other family members, that the clinician should carefully document discussions held with the family and to continue to encourage them to disclose information to those at risk.

Among the considerations of offering genetic testing to other at-risk family members in this pedigree, are the potential legal ramifications of a diagnosis of AIS. In a 1999 case (Littleton vs. Prange) involving a wrongful death suit, a court in Texas ruled that the transsexual woman's marriage to her deceased husband was invalid because of her 46,XY karyotype. This sort of ruling could extend to many areas of the law in which sex is a central issue (eg, discrimination, choice in marriage, participation in sports, housing in higher education and the penal system) and could conceivably affect individuals with common sex chromosome aneuploidy, ie, Turner syndrome and Klinefelter syndrome.

The gender medicine team involved in the present case discussed AIS extensively with the parents. Details of both physical and psychosexual development, specifically gender identity and gender role, were reviewed. Questions were answered and additional information was provided pertaining to child rearing, the rationale for gonadectomy to prevent testicular malignancy, future hormone replacement, infertility issues, and the potential for legal complications to arise. The medical team conducted an assessment of the family's understanding of the condition and its future implications. The family was also made aware of psychologists' availability for support and assistance in discussing the diagnosis with the affected girl at an appropriate age. The parents were also strongly encouraged to disclose the information about AIS to extended family members so that they could seek genetic counseling and testing, if desired.

With regard to DSD, more generally, the authors noted that in the context of current knowledge regarding the process of sex determination and differentiation, unidimensional definitions of "sex" are inherently problematic. They suggested that clinicians be prepared to advocate on behalf of affected patients when caught in legal predicaments, perhaps in the form of an amicus curiae from the American Academy of Pediatrics.

Berg JS, French SL, McCullough LB, et al. Ethical and legal implications of genetic testing in Androgen Insensitivity Syndrome. J Pediatr. 2007;150:434-8.

Editor's Comment: *The reader can find additional guidance regarding the friction between the principles of confidentiality and disclosure of genetic information*

in 2 recent reports.[1, 2] The authors of the current case report do not inform us whether the family in question gave consent to disclose the patient's diagnosis to at-risk extended family members. Regardless of whether they did or not, the "gender team" should be commended for delivering care to the family in a manner consistent with the recent Consensus Statement of Management of Intersex Disorders.[3] The process of disclosing all aspects of the DSD and its clinical care should be collaborative, on-going, and planned with the parents from the time of diagnosis. But, what if the family in this case refuses to allow disclosure to other, potentially affected family members? The 1983 President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research[4] provides some valuable guidance. For example, it states that when the patient refuses, a health care professional's disclosure to at-risk family members should take place only when: (1) reasonable efforts to elicit voluntary consent to disclosure have failed; (2) there is a high probability that harm will occur if the information is withheld, and the disclosed information will actually be used to avert harm; (3) the harm that would result to identifiable individuals would be serious; and (4) appropriate precautions are taken to ensure that only the genetic information needed for diagnosis and/or treatment of the disease in question is disclosed. Approximately 10 years later, the Committee on Assessing Genetic Risks of the Institute of Medicine[5] added an additional criterion: that there is no other reasonable way to avert harm. Neither group implied that the clinician has a legal duty to inform relatives, instead arguing for an ethical duty and legal permission to inform in certain cases.

David E. Sandberg, PhD

References

1. Godard B, Hurlimann T, Letendre M, et al. Fam Cancer. 2006;5:103-16.
2. Wolff K, Brun W, Kvale G, et al. Am J Med Genet. 2007;2:142-8.
3. Lee PA, Houk CP, Ahmed SF, et al. Pediatrics. 2006;118:e488-e500.
4. United States. President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. Screening and Counseling for Genetic Conditions: A Report on the Ethical, Social, and Legal Implications of Genetic Screening, Counseling, and Education Programs. Washington, DC: U.S. Government Printing Office; 1983.
5. Institute of Medicine, Committee on Assessing Genetic Risks. Assessing Genetic Risks: Implications for Health and Social Policy. Washington, DC: National Academy Press; 1994.

CAH Women: Sexual and Reproductive Outcomes

Gastaud et al performed a cross-sectional study using face to face interviews, written questionnaires, the Female Sexual Function Index (FSFI), a brief self-report measure of female sexual function, and a gynecological examination in 35 women aged 18 to 43 years with congenital adrenal hyperplasia (CAH), presenting Prader stages I-V at birth who had been treated from birth to adolescence in the same pediatric endocrine clinic. The objectives of the study were to obtain a detailed description of sexual and reproductive outcomes in adult women with CAH and to compare these outcomes among CAH subtypes and with non-CAH controls. Fourteen of the CAH patients had presented with severe masculinization of their external genitalia at birth (11 with Prader IV and 3 with Prader V stages).

None of the patients expressed doubts about their gender assignment. At gynecological examination cosmetic and anatomic outcomes were considered good by both the patients and the examiner, and 65% of the subjects presented with a satisfactory clitoris, introitus and vagina. However, 9 of 35 patients (26%) were diagnosed with vaginal stenosis, 6 of these belonging to the Prader IV-V group at birth. Seven subjects (20%) reported homosexual inclinations, compared with 5.7% in the control group and 6.6% in a large survey of age-matched women in France (ACSF) and these tendencies were present in 43% (6 of 14) of the Prader IV-V women. A decrease in sexual function was noted when the 35 CAH patients were compared with the 69 healthy controls utilizing the FSFI questionnaire, thus 37% (13 of 35) reported never having sexual intercourse with vaginal penetration by their partners compared with 5% in the ACSF survey. Of these women, 8 attributed their lack of sexual intercourse to the anatomy of their genitalia, 2 believed intercourse would be painful and/or 7 had no partner; the 3 patients born Prader V were among this group. Some degree of pain during vaginal penetration was experienced by 56%, 9 of them presented with moderate or marked stenosis of their introitus. Eight patients cohabited with their partner or were married and 77% wished to be pregnant in the near future or at a later time. Eight subjects became pregnant, only one in the Prader IV-V group; however, only 17% (6 of 35) had children compared to 71% of French women in the ACSF survey. The authors concluded that despite the expert medical and surgical care received by these patients, women with CAH suffer major limitations in their sexual function and their reproductive life.

Gastaud F, Bouvattier L, Duranteau L, et al. Impaired sexual and reproductive outcomes in women with classical forms of congenital adrenal hyperplasia. J Clin Endocrinol Metab. 2007;92:1391-6.

Editor's Comment: *Female neonates with CAH may present with some degree of masculinization of their external genitalia at birth and those with severe virilization (Prader stages IV-V) may require extensive surgery to correct for different degrees of clitoral enlargement and labio-scrotal fusion. In addition, many may develop chronic masculinization as a consequence of being exposed to an excess of adrenal androgens postnatally, with the development of hirsutism, acne, muscle*

hypertrophy and stature, all of which may affect their sexuality and their physical attractiveness.

A number of studies have shown that 46,XX CAH women develop female gender identities,[1,2] but while earlier studies suggested that they had mostly satisfactory sexual intercourse,[3] more recent reports have suggested that they may present with an increased incidence of sexual dysfunction, which seems to be largely related to difficulties in vaginal penetration.[4,5] This seems to be true mainly for those with the most virilized external genitalia at birth, whereas CAH women with a lesser degree of sexual ambiguity at birth seem to have nearly normal sexual outcomes.

While cosmetic and anatomic outcomes of surgery were generally satisfactory to most patients and medical examiners, CAH women, particularly those with Prader IV-V stages, expressed an increased homosexual orientation and a decreased frequency of sexual intercourse. This report and previous studies seem to show that while a large percentage of women with CAH are satisfied with their physical and genital appearance, sexual dysfunction and impaired reproductive outcomes are frequent in this population and will require better medical and particularly surgical care, longer and more detailed follow up, and the transmission of more comprehensive information to parents and/or patients of the risks to sexual function following reconstructive surgery.

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References

1. Meyer-Bahlburg HF. Endocrinol Metab Clin North Am. 2001;30:155-71.
2. Hines M, Brook C, Conway GS. J Sex Res. 2004;41:75-81.
3. Azziz R, Mulaikai RM, Migeon CJ, et al. Fertil Steril. 1986;46:1011-4.
4. Wisniewski AB, Migeon CJ, Malouf MA, et al. J Urol. 2004;171:2497-501.
5. Minto CL, Liao LM, Woodhouse CR, et al. Lancet. 2003;361:1252-7.

Height in Survivors of Childhood Acute Lymphoblastic Leukemia

This paper describes adult height in a Childhood Cancer Survivor Study (CCSS) cohort of 2434 subjects who were at least 5-year survivors of acute lymphoblastic leukemia (ALL) and were diagnosed between 1970 and 1986. Their data were compared to that of 3009 siblings selected for being the closest in age to the proband. Only those over 18 years of age were included. Survivors were excluded from this analysis if they were diagnosed after 17 years of age or if they had a recurrence of their primary leukemia, a secondary malignant neoplasm, or underwent stem-cell transplant before 18 years of age. Cumulative chemotherapy doses were categorized into none, low, medium, or high based on tertiles from previously published end-cut points. For some of the agents dosage information was not available and exposure was recorded as yes or no. Central nervous system (CNS) radiotherapy doses were abstracted in 5-Gy increments. Of the survivors who received cranial radiotherapy, 95% were treated with doses of 15 to 29 Gy and as a result, radiotherapy was characterized into <20 Gy and >20 Gy. Height was expressed in absolute terms as well as SDS. Pubertal status was not always recorded, therefore this variable was dichotomized at age 8 for girls and 10 for boys.

The median age of the study cohort was 27 years, and 51% were female. Median age of the siblings was 31 and 52.7% were female. All survivor treatment groups, including those treated with chemotherapy alone, had decreased adult height and height SDS compared with siblings ($p<0.001$). Effects of radiotherapy on adult height SDS differed between those who were prepubertal versus postpubertal at diagnosis. The height SDS was decreased at all doses of cranial and craniospinal radiotherapy in survivors diagnosed before puberty, compared with those treated with chemotherapy alone. Those survivors who had received >20 Gy of cranial radiotherapy were on average shorter with height SDS scores on average 0.88 lower than those treated with cranial radiotherapy alone. Among survivors diagnosed after pubertal onset, significant negative impact on height SDS was not seen on any cranial radiotherapy dose as compared with chemotherapy alone. On average, the adult height SDS of survivors treated after pubertal onset remained shorter than their siblings. All survivor exposure groups were at significant greater risk of adult short stature (that is height SDS < –2) as compared with siblings. No chemotherapeutic agent analyzed had a consistent dose effect on adult height SDS analyzed individually or in combination. There was an increased proportion of female survivors with adult short stature (12.5%) as compared with male survivors (5.5%).

The authors stated that this report represents the largest cohort of adult ALL survivors evaluated for adult height to date. Significant differences in height outcomes between survivors treated with high doses of cranial radiotherapy as well as those treated with lower dose cranial radiotherapy versus chemotherapy alone were demonstrated. Survivors who received any spinal radiotherapy had the shortest adult heights.

Mechanisms by which cranial radiotherapy affects short stature remain uncertain. It is speculated that at higher doses of radiation there may have been some degree of growth hormone deficiency, especially as it relates to the pubertal growth spurt and peak growth velocity. The second possibility is that cranial radiotherapy exerts its effects on pubertal timing. It would appear that early puberty occurs, especially in females, when treated at an early age. A combination of growth hormone insufficiency and early puberty is certainly associated with short adult stature. Findings in the current study are consistent with this hypothesis, since the risk of adult short stature was greater in those diagnosed at a

younger age, and girls were more affected than boys. The authors pointed out that the limitations of the study included the use of self or proxy reported height data, lack of longitudinal growth information, and the specific time of documentation of pubertal status. However, the large size of the study and the use of sibling controls helped to validate the significance of the differences found. Finally, the authors stated that most patients with ALL were currently treated with chemotherapy alone. Therefore the relationship between chemotherapy agents and linear growth velocity should be available in the future.

Chow E, Friedman D, Yasui Y, et al. Decreased adult height in survivors of childhood acute lymphoblastic leukemia (ALL): A report from the Childhood Cancer Survivor Study. J Pediatr. 2007:150:370-5.

Editor's Comment: *This paper is accompanied by a thoughtful editorial by Oberfield.[1] Her comments included a discussion of previous reports from the CCSS regarding morbidity among childhood cancer survivors and specifically those who were survivors of childhood brain cancers and were subsequently treated with growth hormone. Oberfield points out shortcomings with regard to self reported or proxy reported height and the definition of prepubertal and pubertal based on age, but affirms the uniqueness of the study because of its large size and the fact that even with chemotherapy alone there was a greater than threefold increased risk of decreased stature.*

The data in this study involved survivors of ALL who were treated with a treatment regimen which differs from that currently in use. It clearly demonstrated that previous treatment regimens were associated with reduced adult height. It is hoped that oncologists will continue to carefully record auxologic and pubertal data on their patients so that similar long-term outcomes can be examined from a different therapeutic era in the future.

William L. Clarke, MD

Reference

1. Oberfield SE. J Pediatr. 2007;150:332-4.

Congenital Hypothyroidism—Outcome of Early Treatment

Previous research conducted by Kempers and colleagues, in a cohort born and screened in 1981-1982, demonstrated persistent cognitive and motor deficits associated with congenital hypothyroidism despite initiating T_4 replacement at a median age of 28 days after birth. In the present study, the same investigators examined potential benefits of commencing T_4 replacement at an earlier age (median = 20 days) for a cohort born in 1992 and 1993. During this time, Dutch pediatricians were advised to start with 6-8µg T_4/kg/day with T_4 dose adjustments based on thyroid function labs obtained at regular outpatient follow-up visits.

Participants included 82 Dutch children (mean age 10.5 years, range 9.6 to 11.4 years) diagnosed with thyroidal congenital hypothyroidism (CH-T). An additional 5 participants were diagnosed with central congenital hypothyroidism (CH-C); results were analyzed separately for these due to differing etiology, treatment regimen, and sequelae.

Intelligence was assessed with the Dutch version of the Wechsler Intelligence Scale for Children, third edition (WISC-III), except for the first 10 patients for whom the WISC-R was used (and recalculated into WISC-III scores according to recommended guidelines). Three IQ scores were derived for each participant: full-scale IQ (FSIQ), verbal IQ (VIQ), and performance IQ (PIQ). General population IQ scores for each domain have a mean of 100 (±15). Motor skills were assessed with the Movement Assessment Battery for Children (MABC), designed to identify motor function impairments in children aged 4-12 years, including subscales for manual dexterity, ball skills, and balance; higher scores indicating more motor problems. For the 1981-1982 cohort, motor skills were assessed using a

IQ scores of the CH-T group

	FSIQ	*P* (t)	Verbal IQ	*P* (t)	Performance	*P* (t)
Severe CH-T (n=41)	93.7(89.5-97.9)[1,3]	0.004(–3.0)	94.9(90.1-99.7)[2]	0.039(–2.1)	93.9(90.9-97.8)[1,3]	0.003(–3.1)
Moderate CH-T (n=19)	96.2(88.9-103.5)	0.290(–1.1)	95.4(87.9-102.9)	0.210(–1.3)	98.0(91.1-104.9)	0.550(–0.6)
Mild CH-T (n=22)	105.0(99.5-110.4)	0.73(1.9)	103.6(98.2-109.1)	0.182(1.4)	105.3(99.3-111.3)	0.082(1.8)
Total (n=82)	97.3(94.2-100.4)	0.088(–1.7)	97.4(94.1-100.6)	0.113(–1.6)	97.9(94.8-100.9)	0.172(–1.4)
Range	57-129		65-138		58-134	

IQ scores, expressed as mean (confidence interval), are presented for the total CH-T group and the three severity subgroups.
P values (with t value in *parentheses*) refer to the comparison with the normative population.
[1] $P < 0.01$ compared to the popluation mean
[2] $P < 0.05$ compared to the population mean
[3] $P < 0.01$ compared to mild CH-T

forerunner of the MABC: the Test of Motor Impairment (TOMI). IQ and motor scores were compared among the following subgroups: severe vs. moderate vs. mild CH-T (based on pretreatment free T_4 concentration) and early-treated vs. late-treated patients (ie, before or after the mean starting day of treatment) with severe, moderate, or mild CH-T.

Although mean FSIQ, VIQ, and PIQ scores for the total 1992-1993 cohort were not significantly different from population norms, those in the severe CH-T subgroup received lower scores in all 3 areas (Table). In contrast, IQ scores were not significantly different from the population means for the moderate or mild CH-T subgroups. With regard to motor development, the mean total MABC was significantly poorer than that of the normative population; and a significantly higher proportion of all CH-T severity subgroups received "subnormal" scores. Patients with severe CH-T had significantly worse total MABC and manual dexterity scores than patients with moderate CH-T.

In the severe CH-T group, IQ and motor scores did not differ in patients treated before or later than 19 days after birth. Moreover, IQ and motor scores were not different in the moderate and mild CH-T group when treatment was initiated either before or after 19 and 31 days, respectively. Only the severity of CH-T appeared to be a significant predictor of FSIQ when a multiple regression analysis was conducted using severity of CH-T and starting day of treatment as predictor variables.

Compared to patients from the earlier cohort, those from the 1992-1993 cohort with mild or severe CH-T had initiated T_4 supplementation at a significantly younger age (days 31 and 19 vs. days 68 and 29, respectively). The initial T_4 dose and the FSIQ scores of the subgroups were not significantly different between the 2 cohorts. In patients with mild CH-T, the percentage of patients with a subnormal total motor score was significantly higher in the 1992-1993 cohort; differences were not significant for severe and moderate CH-T. The authors speculated the reason for the increased motor problems scores in the latter cohort may be a result of selecting a measurement tool (the MABC vs. TOMI) exhibiting enhanced sensitivity.

In summary, patients with severe CH-T, whose treatment with T_4 was initiated at a mean age of 19 days after birth, exhibit significant cognitive and motor deficits. Those with mild or moderate CH-T (initiated at a mean age of 31 and 19 days, respectively) had a better prognosis for IQ, but still showed substantial motor deficits. Based on the observed deficits, despite earlier initiation of T_4 treatment, the authors speculated that intellectual and motor development deficits may be the consequence of the hypothyroid prenatal state.

Kempers MJE, van der Sluijs Veer L, Nijhuis-van der Sanden R, et al. Neonatal screening for congenital hypothyroidism in the Netherlands: cognitive and motor outcome at 10 years of age. J Clin Endocrinol Metab. 2007;92:919-24.

Editor's Comment: *In a review of the earlier paper by Kempers and colleagues in GGH,[1] Lanes noted 2 other recent reports of cognitive deficits among those born with severe CH.[2,3] In the current report, IQ deficits were evident among those with severe CH and motor deficits were discernable across all 3 severity subgroups. The American Academy of Pediatrics and other professional societies recently published a clinical report "Update of Newborn Screening and Therapy for Congenital Hypothyroidism,"[4] in which it was acknowledged that those showing signs of prenatal hypothyroidism may evidence more marked cognitive and other impairments; whether these differences, which were characterized as "minor" are preventable by further optimizing postnatal therapy was considered an open question.*

In consideration of the potentially increased vulnerability of children with severe CH despite early and adequate T_4 supplementation, this subgroup should receive particular scrutiny with regard to neurocognitive function. Parental reports of adequate school performance in early years obviously do not rule out specific learning disabilities that, if left undetected, could result in suboptimal academic achievement misattributed to other factors. Finally, when neurocognitive capacity is the clinical outcome of interest, do not assume good adherence to

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recommended T_4 supplementation; in a psychometric and school achievement study of 14-year-olds with CH, identified by newborn screen, approximately 45% had poorly controlled hypothyroidism.[5] Of particular relevance to the issue of cognitive function in these youths, improved hormonal values were accompanied by significant improvements in test results.

David E. Sandberg, PhD

References

1. Growth Genet Horm. 2006;22: epub www.gghjournal.com/volume 22/2/ab17.cfm.
2. Selva KA, Harper A, Downs A, et al. J Pediatr. 2005;147:775-80.
3. Bongers-Schokking JJ, de Muinck Keizer-Schrama S. J Pediatr. 2005;147:768-74.
4. American Academy of Pediatrics, Rose SR; Section on Endocrinology and Committee on Genetics, et al. Pediatrics. 2006;117:2290-303.
5. New England Congenital Hypothyroidism Collaborative. J Pediatr. 1994;124:383-7.

Failure to Thrive: Terminology and Anthropometry

In the February 2007 issue of the *Archives of Diseases in Childhood*, there are 6 articles or perspectives pertaining to one form of aberrant infant growth termed "failure to thrive" (FTT). As Hughes[1] commented—except for infants with obvious disease (eg, cystic fibrosis, celiac disease), the operative definition of "non-organic" FTT in developed societies is not agreed upon, resulting in difficulty in establishing a clear diagnosis and in blurring the divide between a normal extreme and clinical illness; the latter perhaps associated with impaired development. However, a suboptimal nutritional state is usually recognized as one of the hallmarks of this entity.[2] Olsen et al evaluated growth data from 6090 Danish children examined between 1 to 5 weeks of age, 2 to 6 months of age, and 6 to 11 months of age in an effort to establish the prevalence of this growth pattern. Utilizing 7 anthropometric criteria of FTT (Table), they examined the concurrence of these criteria in establishing its presence. In this population of infants, 27% met one or more of the anthropometric criteria at either the earlier (3-6 months) or later (6-11 months) examinations. Only 1.3% of infants met the criterion "weight <80% of median weight for length," and they were a good deal longer than other infants. Twenty-two percent of infants crossed 2 major weight percentiles downward, but they were substantially heavier at birth and throughout the study than were other children with FTT. None of the infants in this study were concordant for all 7 criteria, and approximately 70% of subjects with FTT met only one criterion. Significant under-nutrition, defined as BMI <5th percentile for chronological age, was present in only 2% of children screened. Olsen et al concluded that "... no single measurement ... is adequate to identify nutritional growth delay ... (or) to predict outcomes such as neurodevelopmental or behavioral outcomes." Spencer reached the same conclusion; indeed this investigator stated unequivocally "weight monitoring is not a good screening test for FTT."[2]

Anthropometric Criteria of Failure to Thrive

• Weight <75% of median weight for chronological age (Gomez criterion)
• Weight <80% of median weight for length (Waterlow criterion)
• Body mass index for chronological age <5th centile
• Weight for chronological age <5th centile
• Length for chronological age <5th centile
• Weight deceleration crossing more than two centile lines; centile lines used: 5, 10, 25, 50, 75, 90, 95, from birth until weight within the given age group
• Conditional weight gain=lowest 5%, adjusted for regression towards the mean from birth until weight within the given age group*

* Conditional weight gain was determined by the "thrive index"—the change in weight z-scores between 2 points, from birth to the later age, adjusted for regression to the mean.

Adapted from Olsen EM, et al. Arch Dis Child. 2007;92:109-14.

Emond and co-workers previously examined family, socioeconomic, and prenatal factors that were epidemiologically related to FTT and found that only higher parity (infants born in a 4th or subsequent pregnancy) and small maternal stature (<160 cm) were associated with poor infantile weight gain during the first 9 months of life.[3] They reported that parental postnatal factors associated with FTT as assessed by conditional weight gain of the offspring are maternal age >32 years, height <160 cm, and parity >3; infant characteristics are prolonged breast feeding (>7 months), slow feeding, and ingesting only small amounts of solid food after 6 months of age.

Lucas et al reviewed the literature reporting lay (primarily maternal) views on infant growth and well being. In this population, infant size was primarily utilized as an index of the health and the quality of care provided by the parent(s). While supranormal growth is not of concern, subnormal growth evokes anxiety and fear about the infant and self-recrimination. Wright and Weaver[4] commented that it is essential to differentiate between size (a static measurement) and growth (a dynamic change) when assessing the likelihood of underlying illness in an infant with FTT and that aggressive intervention in the short, thin, normally growing and developing infant is unnecessary.

Olsen EM, Petersen J, Skovgaard AM, Weile B, Jorgensen T, Wright CM. Failure to thrive: the prevalence and concurrence of anthropometric criteria in a general infant population. Arch Dis Child. 2007;92:109-14.

Emond A, Drewett R, Blair P, Emmett P. Post natal factors associated with failure to thrive in term infants in the Avon Longitudinal Study of Parents and Children. Arch Dis Child. 2007;92:115-9.

Lucas P, Arai L, Baird J, Kleijnen J, Law C, Roberts H. A systematic review of lay views about infant size and growth. Arch Dis Child 2007;92:120-7.

Editor's Comment: *Given the many auxologic criteria (Table) for the identification of an infant with FTT and the observation that one criterion is little better than another, it appears that this diagnosis falls into those typified by "I can't define it, but I know it when I see it." The critically essential finding in most of these subjects is that despite a poor appetite, relatively restricted caloric intake, and low weight for stature, linear growth rate remains normal. (Indeed, this pattern of growth is the diametric opposite of the voracious infant/child who steadily gains weight and crosses weight and height percentiles!) It is particularly important not to designate the normal, slowly growing or small child as abnormal, both because of the need to avoid unnecessary diagnostic and therapeutic interventions as well as to support the parents' confidence and sense of competence to care for their child and to avoid a misplaced charge of negligence. Clearly, the clinician needs to know not only her/his patient but also the child's parents. The criterion for FTT of downward crossing of weight percentiles certainly reflects in most subjects normal variations of growth as such changes are indeed quite frequent.[5] It is of interest that the term FTT has been adopted by the geriatricians to denote "an elderly patient who undergoes a process of functional decline, progressive apathy and a loss of willingness to eat and drink that culminates in death."[1] Were there such a precise definition for the pediatric population, the identification and management of such children would be far more precise.*

Allen W. Root, MD

References

1. Hughes I. Arch Dis Child. 2007;92:97-8.
2. Spencer NJ. Arch Dis Child. 2007;92:95-6.
3. Blair PS, Drewett RF, Emmett PM, et al. Int J Epidemiol. 2004;33:839-47.
4. Wright CM, Weaver LT. Arch Dis Child. 2007;92:98-100.
5. Mei Z, Grummer-Strawn LM, Thompson D, et al. Pediatrics. 2004;113:e617-27.

GH and GnRHa Therapy for Short Stature

This study assessed the final height (FH) and adverse effects of combined growth hormone (GH) and gonadotropin-releasing hormone agonist (GnRHa) treatment in short adolescents with relatively early puberty. Van Gool et al studied 32 adolescents born small for gestational age or with normal birth size, in Tanner stage 2-3, with age and bone age of <12 years for girls and <13 years for boys. Subjects had a height SDS of either <–2 SDS or between –1 and –2 SDS and a predicted adult height (PAH) of <–2 SDS. Patients were randomly allocated to receive GH and GnRHa (n=17) or no treatment (n=15) for 3 years; FH was determined at the age of 18 years or older in girls and 19 years or older in boys.

The FH was not different between treated and untreated subjects. However, treated patients had a greater height gain (FH minus PAH at the beginning of treatment) than the untreated patients (4.4 ± 4.9 vs. –0.5 ± 6.4 cm, respectively; $p<0.05$). Of the treated and control subjects, 76 (60%) had a FH that was greater than the PAH. A significant gain in PAH of 9.3 cm after 3 years of combined therapy was noted in the treated group compared with a 1.2 cm gain in the untreated group. However, during the period of time between treatment discontinuation and FH, 50% of the PAH gain during treatment was lost, resulting in a mean height gain of 4.9 cm (range of –4 to 12.3 cm). Although, treatment did not seem to affect BMI or hip bone mineral density (BMD), the mean lumbar spine BMD and the bone mineral apparent density (BMAD) tended to be lower in treated males. The authors concluded that given the expensive and intensive treatment regimen and the modest height gain attained, as well as the possible adverse effect of therapy on bone mineralization in males, GH and GnRHa treatment cannot be considered for routine treatment of short stature in children entering into early puberty. However, treatment could be considered in children, particularly females, with extremely low adult height prediction, early pubertal onset, and considerable psychosocial problems.

Van Gool SA, Kamp GA, Visser-van Balen H, et al. Final height outcome after three years of growth hormone and gonadotropin-releasing hormone agonist treatment in short adolescents with relatively early puberty. J Clin Endocrinol Metab. 2007;92:1402-8.

Editor's Comment: *The final height of short children entering into puberty at an early age may be quite limited due to premature epiphyseal fusion induced by the early secretion of gonadal steroids. Treatment with GnRHa to delay or halt pubertal onset has been attempted in this group of patients, but the growth velocity of some of them has been noted to decrease to levels below the normal pubertal velocity, possibly as a result of accelerated growth plate senescence induced by previous estrogen exposure. GH treatment in short children with idiopathic short stature or born small for gestational age has been shown to increase final height, particularly if begun at an early age. Combined GH and GnRHa therapy in short children entering into puberty at an early age has been attempted in several studies with a height gain of between 1 to 10 cm and the effectiveness of this form of therapy remains controversial. Treatment response has been generally analyzed by comparing treated patients to patients treated only with GH, to an untreated group not randomly assigned, or to no controls at all; most studies included only females.*

Treatment of short, but otherwise healthy children with medications that require parenteral administration, close

supervision, frequent laboratory testing, and are extremely expensive, should only be considered if the height gain obtained is significant and if the medications are proven to be safe. As clearly stated by the authors, the costs of this form of therapy seem to overshadow the modest benefit in height gain obtained; therefore this form of therapy should not be recommended for routine use in short but otherwise healthy patients who enter into puberty at an early age.

Roberto Lanes, MD

References

1. Weise M, Flor A, Barnes KM, et al. J Clin Endocrinol Metab. 2004;89:103-7.
2. Nilsson O, Baron J. Trends Endocrinol Metab. 2004;15:370-4.
3. Wit JM, Rekers-Mombarg LT, Cutler GB, et al. J Pediatr. 2004;146:45-53.
4. Balducci R, Toscano V, Mangiantini A, et al. J Clin Endocrinol Metab. 1995;80:3596-600.
5. Lanes R, Gunczler P. Clin Endocrinol (Oxf). 1998;49:197-202.
6. Pasquino AM, Pucarelli I, Roggini M, et al. J Clin Endocrinol Metab. 2002;629:619-22.

GH Inhibition of IGF-I in STAT5b Expression

Ligand binding of the growth hormone (GH) receptor activates, via the Jak2 tyrosine kinase, the Stat transcription factors and the MAP kinase and PI3 kinase/Akt pathways. As is well known to the readers of GGH, GH-stimulated transcription of the insulin-like growth factor (IGF)-I gene requires the Jak2/Stat5b mechanism. However, GH signaling also leads to transcriptional repression of a cohort of genes, including the IGF binding protein (IGFBP)-1. Ono et al sought to elucidate the mechanism of this facet of GH action.

Hypophysectomized Sprague-Dawley rats were given a single systemic pulse of GH, and hepatic RNA was isolated 30, 60 or 120 minutes afterwards. By both microarray and RT-PCR methods, GH acutely increased the mRNA levels of IGF-I and Socs-2 while decreasing that of IGFBP-1. GH also acutely induced the nuclear accumulation of phosphorylated Stat5b. Adenoviral-mediated delivery of a constitutively active Stat5b construct to livers of GH-deficient rats similarly increased IGF-I and Socs-2 expression while decreasing IGFBP-1.

To further examine the transcriptional regulation of IGFBP-1, Cos-7 cells were transiently transfected with a rat IGFBP-1 promoter-luciferase reporter construct as well as an expression vector for mouse GH receptor. Cotransfection with wild-type or constitutively activated FoxO1, a transcription factor important for *IGFBP-1* expression, stimulated promoter activity. GH treatment altered neither IGFBP-1 promoter activity nor the abundance of the FoxO1 proteins. In contrast, when wild type Stat5b was also co-transfected, GH treatment led to a 35%-50% reduction of IGFBP-1 promoter activity with either type of FoxO1; GH stimulated posphorylation of the wild-type but not constitutively activated FoxO1, and abundance of the FoxO1 proteins again were not altered. Thus, GH-induced IGFBP-1 repression is mediated by Stat5b and not Akt (the constitutively activated FoxO1 is Akt resistant.)

Because IGFBP-1 expression is also repressed by insulin, which acts via Akt inhibition of FoxO1, the authors sought to further examine the interactions between Akt, Stat5b and FoxO1. A tamoxifen-inducible Akt fusion protein, iAkt, repressed IGFBP-1 promoter activity in the presence of wild type, but not a constitutively activated, FoxO1; the former form of FoxO1 was phosphorylated by Akt while the latter cannot be. In contrast, a constitutively activated Stat5b did not phosphorylate FoxO1.

Further experiments were performed to mechanistically examine Stat5b inhibition of FoxO1. Using a luciferase reporter construct driven by a minimal promoter containing 3 copies of IRSA (one of the tandem FoxO1 binding sequences found in the IGFBP-1 promoter), the FoxO1 binding site was shown sufficient for GH and Stat5b inhibition of FoxO1-stimulated gene transcription. To examine the possibility of reciprocal inhibition, a luciferase reporter construct driven by the Stat5b-dependent HS7 response element (found in the IGF-I gene) was examined. It increased activity in response to GH in the absence of FoxO1, and increased further still when wild type or constitutively activated FoxO1 were cotransfected, even though there were no FoxO1 binding sites in the HS7-promoter sequences. Thus, competition for transcriptional co-factors does not seem to be the mechanism of Stat5b's inhibition of FoxO1 activity. A dominant-negative Stat5b was shown to lose the ability to mediate GH inhibition of IGFBP-1 promoter activity, in both co-transfected Cos-7 cells in vitro and in GH-treated hypophysectomized rats in vivo. Co-transfected Cos-7 cells further showed that GH induced nuclear accumulation of Stat5b, but neither nuclear levels of FoxO1 protein nor its DNA-binding ability were reduced by activated Stat5b. Direct protein-protein interactions between FoxO1 and Stat5b from Cos-7 nuclear extracts were not detected by co-immunoprecipitation assays or avidin-biotin complex DNA binding assay.

Finally, the authors returned to their hepatic microarray results from GH-stimulated hypophysectomized rats. They compared the list of GH-repressed genes to genes repressed by adenovirally introduced constitutively activated Stat5b. Eighty-nine gene transcripts were similarly reduced by both mechanisms. *In silico* search for FoxO1 binding sites within phylogenetically conserved (rat and human) regions of these genes revealed 19 hits, or 21% of the repressed genes. Of 322 randomly selected genes not regulated by GH or Stat5b 19% were also found to contain FoxO1 binding sties. Thus, FoxO1 inhibition accounts for only a subset of transcriptional repression by GH/Stat5b.

Ono M, Chia DJ, Merino-Martinez R, Flores-Morales A, Unterman TG, Rotwein P. Signal transducer and activator of transcription (stat) 5b-mediated inhibition of insulin-like growth factor binding protein-1 gene transcription: a mechanism for repression of gene expression by growth hormone. Mol Endocrinol. 2007;21:1443-57.

Editor's Comment: *Through a well constructed series of experiments, Ono et al clearly showed that GH inhibits IGFBP-1 expression via activated Stat5b and FoxO1. However, the exact mechanism of FoxO1 inhibition by Stat5b remains elusive; FoxO1 protein degradation, nuclear exclusion and impaired DNA binding ability were all ruled out, as was direct protein-protein interaction between Stat5b and FoxO1. Nonetheless, this paper expands our thinking along 2 lines. First, GH, via activated Stat5b, not only induces gene expression (eg. IGF-I), but also represses transcription of other genes, such as IGFBP-1. Thus, the genetic response to GH/Stat5b signaling is a richer compilation of coordinated alterations than previously appreciated. Second, the mechanism whereby IGFBP-1 expression is repressed by GH is clearly distinct from that of insulin (activated Akt phosphorylating FoxO1, thereby sequestering it out of the nucleus and impairing its ability to transcribe IGFBP-1[1]). Although we are used to thinking of GH as counter-regulatory to insulin, in certain circumstances, like IGFBP-1 expression as shown here, the two hormones can act synergistically because they effect the same molecular change through separate pathways.*

Adda Grimberg, MD

Reference

1. Guo S, Rena G, Cichy S, et al. J Biol Chem. 1999;274:17184-92.

GH Neurosecretory Dysfunction and Cranial Irradiation

The group of Shalet in Manchester, UK has made fundamental contributions to the understanding of the broad range of endocrinopathies which may follow cancer therapy in children. In terms of clinical practice, deficiency of growth hormone (GH) following cranial irradiation constitutes an important entity of which all pediatric endocrinologists need to be aware. Prophylactic cranial irradiation for leukemia has been largely replaced by use of intrathecal cytotoxic agents. However, targeted high-dose radiotherapy (RT) for brain tumors outside the hypothalamic pituitary region, such as medulloblastomas, remains an essential and potentially life-saving therapy.

The relationship between the dose of RT and the frequency of subsequent GH deficiency has been clearly established. This article critically considers whether patients who have normal GH responses to pharmacological testing may have a more subtle defect of physiological pulsatile GH release, ie, so-called GH neurosecretory dysfunction. The presence of this 'defect' of probably hypothalamic origin was assumed when subnormal pulsatile secretion was reported during adolescence, particularly after low-dose RT in several studies.

Darzy KH, Pezzoli SS, Thorner M, Shalet SM. Cranial irradiation and growth hormone neurosecretory dysfunction: A critical appraisal. J Clin Endocrinol Metab. 2007;92:1666-72.

Editor's Comment: *The combined groups of Shalet and Thorner have performed extremely detailed assessments of physiological GH secretion (cluster analysis) in adult patients, most of whom received RT during childhood, and in normal controls. Such a study would have been impossible in pediatric subjects. The hallmarks of neurosecretory dysfunction, ie, normal GH secretion, after provocation compared with decreased spontaneous secretion were not seen. This helpful finding effectively dismisses this abnormality from potential sequelae of cranial RT in childhood. The peak GH concentration after a pharmacological provocation test can be taken as a realistic index of somatotrope secretory capacity. Performing physiological studies is unlikely to add further clinically relevant information.*

Martin O. Savage, MD

Growth in Treated Classical Galactosemia Patients

Panis and co-workers studied height and weight growth over a period of 2 years in a group of 40 Dutch children and adolescents with classical galactosemia. These subjects (13 boys, 27 girls, median age 7.8 years, range 3 to 17 years) had the diagnosis established in the neonatal period by galactose-1-phosphate-uridyltransferase (GALT) and enzymatic studies in erythrocytes. Of the 40 subjects, 31 were prepubertal, and 5 had reached Tanner stage 5. Urinary galactose and galactitol concentrations and GALT levels in the erythrocytes were measured during the study and all were within the range of treated patients. Prenatal growth was evaluated by obtaining length, weight, and head circumference data from infant welfare centers or from parents. The results, corrected for gestational age, were within normal limits for the Dutch population. Yearly, for 2 successive years, postnatal growth was evaluated by z-scores and corrected for target age. Mean height growth velocity was 0.87±1.2 (range −0.4 to 3.6) for boys and −0.89±2.1 (range −2.5 to 3.7, $p=0.047$) for girls. Weight growth velocity in z-scores was 0.91±1.6 (range −0.8 to 4.2) for boys and −0.74±1.3 (range −3.1 to 2.3, $p=0.008$) for girls. Mean

height in z-scores corrected for target height z-scores was decreased in both genders with girls being more affected than boys. Height velocities were correlated with insulin like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 z-scores and with the height z-scores corrected for target.

The authors affirmed normal prenatal growth in boys and girls with galactosemia, but decreased height and weight growth velocities. In addition they stated that predicted final height was less than target height in most patients after birth. The authors' review of the literature suggested a variety of variable findings in at least 3 other studies, some showed decreased height-for-age but final height within normal limits,[1] microcephaly,[2] and reduced birth weight in affected neonates.[3] The authors speculated that possible risk factors for abnormal growth include either intrinsic or diet-related factors, decreased mean IGF-I and IGFBP-3 concentrations and/or hormonal factors.

Panis B, Gerver W, Rubio-Gozalbo ME. Growth in treated classical galactosemia patients. Eur J Pediatr. 2007;166:443-6.

Editor's Comment: *Galactosemia may be a more common finding in genetics clinics than in endocrine clinics. The growth data which Panis reported in a large group of children with classical galactosemia would not usually result in a referral to a pediatric endocrinologist for evaluation. It would have been interesting had these investigators provided a little more information especially in regard to how they determined predicted adult height. There is no mention of bone ages being performed in these individuals. It is easy to speculate that girls with galactosemia and ovarian dysfunction would most likely have lower height z-scores than the normal population. Despite its shortcomings, this paper presented important information which suggests when children with classical galactosemia are evaluated in either genetics or metabolic clinics, there should not be an expectation for short stature or failure to thrive, at least when the diet is followed consistently. Thus short children with classical galactosemia should be evaluated thoroughly for other hormonal causes of growth failure.*

William L. Clarke, MD

References

1. Waggoner DD, Buist NR, Donnell GN. J Inherit Metab Dis. 1990;13:802-18.
2. Schweitzer S, Shin Y, Jakobs C, et al. Eur J Pediatr. 1993;152:36-43.
3. Hsia DY, Walker FA. J Pediatr. 1961;59:872-83.

Height Screening During the Primary School Years: Evidence Behind Practice?

Height and weight monitoring has long been a fundamental aspect of pediatric care as indicators of both health and possible underlying pathology. Unfortunately, delays in diagnosis and treatment of underlying growth problems are frequently observed. The optimal strategy remains elusive, as the standard cut-offs between normal and abnormal and the recommended growth screening practices vary widely. For example, the Child Health Subcommittee of the UK National Screening Committee recommended a cut-off of 0.4th centile and a single height and weight measurement at or around the time of school entry for screening.[1]

Fayter et al performed a systematic review of the effectiveness and economic modeling of height screening in primary school aged children to identify height-related conditions (focusing on stature, not obesity). They collected all studies from database inception (1974) to July 2005 that measured child height as part of a population-level assessment of children aged 4 to 11 years in Western Europe, North America, Australia and New Zealand (excluding aboriginal populations). All study designs, except case reports, were accepted.

Effectiveness was assessed from the number of cases of all conditions detected. Meta-analysis of diagnostic yield data was precluded by the heterogeneity of child age, reference charts and screening methods used; thus, effectiveness data were limited to descriptive summaries. Twelve studies of height screening programs provided diagnostic yields of new cases and measured 45% to 90% of eligible children. A single measurement at school entry identified new cases of underlying growth conditions at rates of 0.54 to 0.56 per 1000 children screened.

Economic modeling was based on pooled raw data from 12 diagnostic yield studies, providing probability distributions for new case detection of each included condition. Lifetime costs and outcomes were modeled, following NICE guidelines, and included screening, referral, and treatment costs reflated to 2006 values. A cost/QALY analysis (a QALY = a year of life, adjusted for its quality or perceived value) compared height screening at school entry (age 5 years) versus no screening (diagnoses found later in clinical practice). QALY estimates, based on the literature and an expert clinical panel, assumed early detection and screening would provide double the QALY gains than later detection from no screening. Using the number of 5-year-old children in England and Wales, the model found an incremental cost-utility of height screening at £9,900 (~$19,800 US) per QALY. Probabilistic sensitivity analysis found that all of the model's data distributions fell below the UK willingness to pay thresholds of £30,000 per QALY. Thus, the authors concluded that height screening in primary school aged children is diagnostically useful and economically justifiable.

Fayter DA, Nixon J, Hartley S, et al. Effectiveness and cost-effectiveness of height screening programmes during the primary school years: a systematic review. Arch Dis Child. 2007 May 2. [Epub ahead of print]

Editor's Comment: *It is striking that such financial analyses are now needed to justify growth screening, a fundamental tenet of pediatric care. However, as highlighted by this paper, many of the considerations remain elusive. What is the optimal height cut-off to identify likely pathology? What is the optimal screening paradigm? Serial height measurements will capture cases of growth deceleration before they become severe enough to cross the single height cut-off for pathology, but how frequent and how many are needed to balance improved sensitivity with increased cost? What is the actual cost of missed or delayed diagnoses and how are QALYs estimated, especially since the impact of short stature on quality of life remains so controversial? And what about the cost of height monitoring itself? Height measurements in the United States are performed as part of routine pediatric well child care,[2] and the cost of a stadiometer spread across the patient population is so negligible that it seems virtually free. The only real cost is the time to accurately measure the child and plot the measurements on the appropriate growth chart. With the increasing pressures to expedite patient flow faster and faster, time may be the most expensive aspect of growth screening.*

Adda Grimberg, MD

References

1. National Screening Committee. Child health sub-group report: growth disorders. Leeds: National Screening Committee; 2004.
2. American Academy of Pediatrics Policy Statement - Committee on Practice and Ambulatory Medicine. Pediatrics. 2000;105:645.

Histrelin Subdermal Implant in Central Precocious Puberty

This important article describes efficacy and safety data related to the use of a single annual subcutaneous implantation of a gonadotropin-releasing hormone analogue (GnRHa) to induce pituitary gonadotropin suppression in children with central precocious puberty. Histrelin provides a continuous slow release at an average rate of 65 µg/d of GnRHa. Its use as a single yearly implant has previously been shown to effectively suppress LH, FSH and testosterone secretion in adult males with prostate cancer.[1,2] This report is the first in children with precocious puberty.

The procedure of implantation will require more detailed examination with wider clinical use. A pediatric surgeon is required to perform this procedure and in this study, local or general anesthetic or sedation was used. There is no comment about any practical difficulties with the implantation in terms of interference with daily activities such as sports and recreation, or whether the implant became dislodged in some patients.

Eugster EA, Clarke W, Kletter GB, et al. Efficacy and safety of histrelin subdermal implant in children with central precocious puberty: A multicenter trial. J Clin Endocrinol Metab. 2007;92:1697-704.

Editor's Comment: *The data on sex steroid, LH and FSH evaluation are impressive and clearly show that effective suppression of gonadotrope function occurs for 12 months after a single subcutaneous implantation. The choice of patients may need to be individualized and an implantation technique which avoids general anesthetic would clearly be preferable. Longer term studies to assess recovery of the pituitary-gonadal axis following discontinuation of treatment are important. This first report in children is encouraging and may eliminate the discomfort of monthly or three-monthly injections as currently practiced.*

Martin O. Savage, MD

References

1. Schlegel PN, Kuzma P, Frick J, et al. Urology. 2001;58:578-82.
2. Chertin B, Spitz IM, Lindenberg T, et al. J Urol. 2000;163:838-44.

Hypogonadotropic Hypogonadism—Mutations and Phenotypes

Isolated hypogonadotropic hypogonadism (IHH) has been associated with mutations in 7 genes to date (Table). The products of the genes encoded by *KAL1, FGFR1, PROK2, PROKR2*, and *NELF* assist in the regulation of neural movement within the CNS—particularly the migration of olfactory and gonadotropin releasing hormone (GnRH)-containing neurons from the olfactory placode during early embryogenesis. Mutations in these genes result in abnormalities of GnRH secretion and the reproductive endocrine system (delayed adolescence, hypogonadotropism) and the sense of smell (hyposmia, anosmia), and those afflicted often display other neurologic (bimanual synkinesia) and somatic (renal agenesis) anomalies. These traits are transmitted in an autosomal dominant manner often with incomplete penetrance and substantial inter- and intrafamilial variability in clinical manifestations. Mutations of *GPR54* limit release of GnRH while those of the *gonadotropin-releasing hormone receptor (GNRHR)* impair its function at the gonadotroph membrane. These disorders are transmitted in an autosomal recessive manner and are not associated with other specific clinical or anatomic abnormalities.

Intrigued by the variable clinical manifestations of IHH, Pitteloud and colleagues examined the genotype of 2 families in which single gene defects thought to have resulted in IHH had been previously identified. In pedigree #1, a 21-year-old male with IHH and

Table: Genetic causes of isolated hypogonadotropic hypogonadism.

Gene	Locus	Gene product	OMIM
KAL1	Xp22.2	Anosmin (KAL1)	308700
FGFR1	8p11.2-p11.1	Fibroblast growth factor receptor 1 (KAL2)	136350
PROK2	3p21.1	Prokineticin 2 (KAL4)	607002
PROKR2	20p13	Prokineticin receptor 2 (KAL3)	607123
NELF	9q34.3	Nasal embryonic luteinizing hormone-releasing factor	608137
GPR54	19p13.3	G-protein coupled receptor 54	604161
GNRHR	4q21.1	Gonadotropin-releasing hormone receptor	138850

hyposmia was initially found to have a heterozygous mutation in *FGFR1* (Ser342Leu—chromosome 8p11.2-p11.1); the proband's father and sister had the same *FGFR1* mutation; the father had delayed onset and the sister normal timing of puberty. In vitro studies demonstrated that the Ser342Leu mutant of *FGFR1* acted in a dominant-negative manner. A heterozygous 8 bp deletion in the negative elongation factor (*NELF*) resulting in a truncated product was later identified in the proband, his mother and his brother; the latter 2 subjects underwent normal puberty. The authors suggested that loss of a single copy of *FGFR1* resulted in a less severe phenotype than did loss of a single copy (allele) of both *FGFR1* and *NELF*. In pedigree #2, two sisters with IHH (no evident spontaneous ovarian function) were found to have inactivating mutations in both *GNRHR* alleles (Gln106Arg, Arg262Gln—chromosome 4q21.2) ie, the sisters had compound heterozygosity. Their father had a history of delayed puberty and carried the Arg262Ser mutation, while their normal mother carried the Gln106Arg mutation. Further studies revealed that the sisters also had a loss-of-function heterozygous mutation in *FGFR1* (Leu470Arg), an allele that they had inherited each from their father. Thus, these sisters with a severe IHH phenotype were triallelic for this trait. Why the father who was heterozygous for mutations in both *GNRHR* and *FGFR1* manifested only delayed puberty is uncertain. The investigators concluded that disorders thought to be monogenic in origin and that manifest variable degrees of clinical involvement may actually be oligogenic due to the involvement of 2 (possibly even more) different genes whose mutations sum to produce the clinical phenotype.

Pitteloud N, Quinton R, Pearce S, et al. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. J Clin Invest. 2007;117:457-63.

Editor's Comment: *A gene mutation has been found in only 30% of patients with IHH. Other genes that regulate migration of GnRH neurons and synthesis and release of or response to GnRH await identification. Clearly the concept of digenic inheritance of disease is one that may well be applicable to many disorders of the endocrine and other systems.*

Allen W. Root, MD

IGF-I Allele in Small Size Dogs

Intrigued by the great diversity in size among the dog family (Canidae), these investigators first identified by genome-wide scan a skeletal size-related quantitative trait locus (QTL) on chromosome 15 within a single breed—the Portuguese water dog (PWD), a breed with great inter-individual variation in size. They next examined the relationship of single-nucleotide polymorphisms (SNPs) within this QTL to skeletal size in large and small Portuguese water dogs. They found one such SNP in this QTL to be associated with size that was near the gene encoding insulin-like growth factor-I (*IGF1*). Designating the haplotypes I and B, the investigators found that Portuguese water dogs homozygous for haplotype I were larger in size and had higher serum IGF-I concentrations than did dogs that were homozygous for haplotype B; they calculated that 15% of the variability of skeletal size within this breed could be accounted for by this *IGF1* haplotype (Figure 1). Performing the same SNP analyses in more size-homogeneous small (n=23, <9 kg) and giant (n=20, >30 kg) canid breeds, the authors found skeletal size to be related to an *IGF1* haplotype characterized by 20 SNPs that was shared by all small breed dogs (and one in particular designated SNP 5 A) (Figure 2). Sequencing of *IGF1* revealed a SNP in exon 3 and several

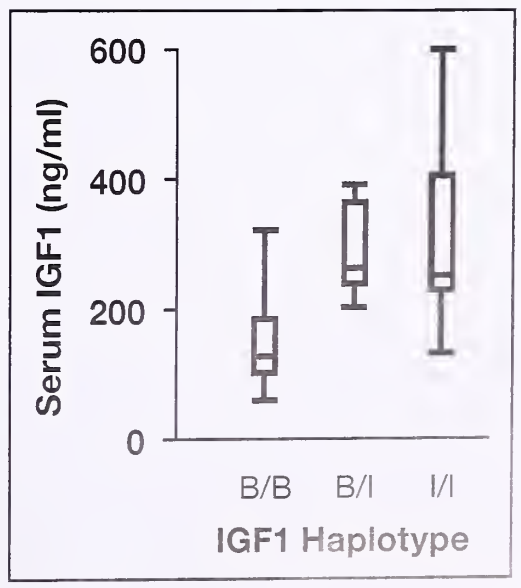


Figure 1. Serum levels of IGF1 protein (ng/ml) as a function of haplotype. Serum levels of IGF1 protein were assayed in 31 PWDs carrying haplotypes B and I. Box plots show the median (center line in box), first and third quartile (box ends), and maximum and minimum values (whiskers) obtained for each category: homozygous B/B ($n = 15$), heterozygous B/I ($n = 7$), and homozygous I/I ($n = 9$).

SNPs in flanking genomic sequences (promoter region) and introns that were unique to small breeds but no specific variant related to size was definitely identified.

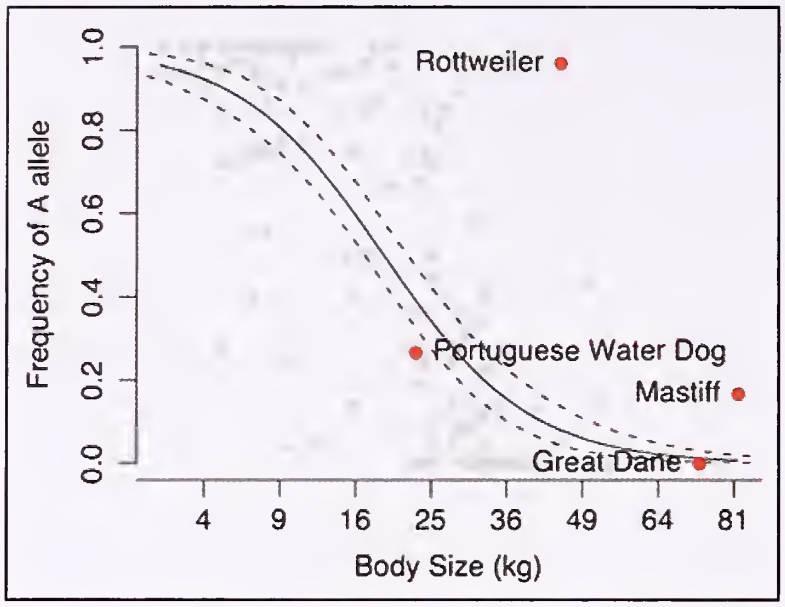


Figure 2. Association of body size and frequency of the SNP 5 A allele. Binomial regression of allele frequency on square root of mean breed mass. Dashed lines indicate the 95% confidence interval on the predicted equation line as estimated from nonparametric bootstrap resampling. Between 5 and 109 (median = 22) dogs were genotyped for each of 143 breeds. The PWD is highlighted in red along with three giant breeds that have larger breed average masses than is predicted by their SNP 5 allele frequency. Reprinted with permission from Sutter NB, et al. Science. 2007;316:112-5. Copyright © AAAS 2007. All rights reserved.

The authors concluded that "a narrow ... genomic region holds the variant ... (in *IGF1*) ... responsible for ... size in a disparate set of small ... (and giant) ... dog breeds ... "

Sutter NB, Bustamante CD, Chase K, et al. A single IGF1 allele is a major determinant of small size in dogs. Science. 2007;316:112-15.

Editor's Comment: *Although previous studies have identified a relationship between serum levels of IGF-I in various dog breeds and have been related to growth in humans, the fact that it is tissue and not serum IGF-I values that determine growth must be remembered.[1] The findings in this report should in no way be construed or utilized to support the use of recombinant human (rh) IGF-I in the treatment of children with idiopathic short stature, a contentious practice.[2] The use of rhIGF-I is of limited value in patients with severe IGF-I deficiency due to growth hormone (GH) resistance due to inactivating mutations of the genes encoding the GH receptor or STAT5 or due to development of neutralizing antibodies to rhGH; it is not indicated nor particularly efficacious in other short stature children while exposing them to significant risks.*

Allen W. Root, MD

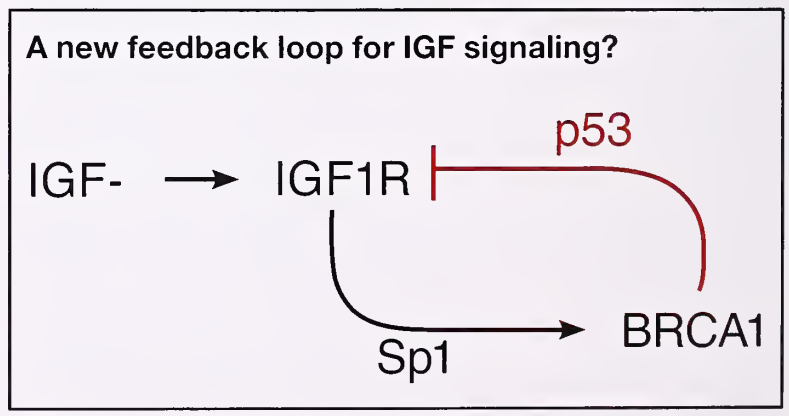
References

1. Yakar S, Liu JL, Stannard B, et al. Proc Natl Acad SciUSA. 1999;96:7324-9.
2. Rosenbloom AL. J Pediatr. 2007;150:7-11.

IGF-I and BRCA1: A New Feedback Loop?

The growth hormone (GH)/insulin-like growth factor (IGF) system plays an important role in normal breast physiology and carcinogenesis. GH receptor (GH-R),[1] IGF-I and type 1 IGF receptor (IGF1R) knock-out mice show impaired mammary ductal development from reduced proliferation in the terminal end buds.[2] Conversely, transgenic mice over-expressing human (h)IGF-I or hIGF-II have reduced apoptosis and hence, delayed breast involution that normally occurs with the cessation of suckling and lactation.[2] Further, dysregulated GH/IGF signaling has been implicated in breast cancer, a subject extensively reviewed elsewhere.[3,4]

Maor et al therefore sought to investigate the regulatory relationship between gene expression of IGF1R and the breast and ovarian cancer susceptibility gene (BRCA1), a major tumor suppressor in breast carcinogenesis. As indicated by Western immunoblotting and RT-PCR, BRCA1 expression was induced by treating MCF-7 breast cancer cells in vitro with IGF-I or IGF-II. Using BRCA1 promoter-luciferase reporter constructs, IGF-I treatment of MCF-7 and BRCA1-null HCC1937 breast cancer cells significantly enhanced promoter activity of the full-length BRCA1 promoter but not a minimal BRCA1 promoter deletion construct that lacks binding sites of the transcription factor Sp1. Drosophila-derived, Sp1-null Schneider cells were then co-transfected with the BRCA1 reporter construct and an Sp1 expression vector, which led to an almost 12-fold increase in BRCA1 promoter activity. Conversely, Mithramycin A, an Sp1-inhibitor, inhibited the IGF-I-stimulated BRCA1 expression and promoter activity in MCF-7 cells. Likewise, siRNA against Sp1 markedly reduced BRCA1 protein levels in MCF-7 cells. Binding of Sp1 to the BRCA1 promoter, as indicated by chromatin immunoprecipitation (ChIP) assay, was enhanced by IGF-I treatment of the MCF-7 cells. Finally, transfection of an anti-BRCA1 siRNA, versus a scrambled siRNA, increased the proportion of MCF-7 cells arrested at SubG0 and reduced those at the G2/M phase in response to IGF-I treatment.



As shown by this paper (black), IGF1R signaling induces BRCA1 gene expression via Sp1. As previously shown (red), BRCA1 represses IGF1R via p53.

The authors concluded that BRCA1 is a novel downstream target of IGF1R signaling. IGF1R signaling induces BRCA1 gene expression via the Sp1 transcription factor, and BRCA1 gene silencing stunted IGF-stimulated cell cycle progression. Thus, they inferred that aberrant IGF signaling may lead to dysregulated BRCA1 expression during breast cancer pathogenesis.

Maor S, Papa MZ, Yarden RI, et al. Insulin-like growth factor-I controls BRCA1 gene expression through activation of transcription factor Sp1. Horm Metab Res. 2007;39:179-85.

Editor's Comment: *BRCA1 is major tumor suppressor involved in breast carcinogenesis, including both somatic dysfunction and increased familial cancer risk due to germline inactivating mutations. Normally, BRCA1 plays a role in genomic stabilization, inducing cell cycle arrest and DNA repair in response to DNA damage.[5] BRCA1 acts as transcription factor, interacting with co-repressors and co-activators, to inhibit expression of growth-promoting genes and stimulate expression of cell cycle arrest and DNA repair genes, DNA damage inducible genes and interferon inducible genes.[6] As shown by the same authors as the current paper, one of the genes whose transcription is repressed by BRCA1 is IGF1R.[7] Thus, their 2 findings may form a feedback loop (Figure), whereby IGF1R signaling induces BRCA1 transcription which in turn represses IGF1R transcription.*

Adda Grimberg, MD

References

1. Kelly PA, Bachelot A, Kedzia C, et al. Mol Cell Endocrinol. 2002;197:127-31.
2. Marshman E, Streuli CH. Breast Cancer Res. 2002;4:231-9.
3. Laban C, Bustin SA, Jenkins PJ. Trends Endocrinol Metab 2003;14:28-34.
4. Gross JM, Yee D. Cancer Metastasis Rev. 2003;22:327-36.
5. Gudmundsdottir K, Ashworth A. Oncogene. 2006;25:5864-74.
6. Mullan PB, Quinn JE, Harkin DP. Oncogene. 2006;25:5854-63.
7. Sarfstein R, Maor S, Reizner N, et al. Mol Cell Endocrinol. 2006;252:241-6.

Intrauterine Growth Retardation and Pituitary Gonadal Function

Low birth weight as a consequence of intrauterine growth retardation (IUGR) is associated with an increased risk of disease in adult life. It has been reported to have a detrimental effect on gonadal development in boys, including cryptorchidism and hypospadias. Little is known on the male pituitary-gonadal axis functioning in adulthood. Small for gestational age (SGA) is a result of IUGR during variable periods of gestation, hence a consequence of different adverse events occurring during gestation. This study focused on fetal growth restraint occurring during the third trimester of pregnancy; the authors hypothesized that IUGR in the third trimester of pregnancy would determine the ultimate male reproductive function. Jensen also evaluated the influence of birth weight in relation to gestational age on the pituitary-testicular axis. Participants were recruited from a large prospective study of pregnant women who provided third trimester fetal growth velocity and birth weight. Fifty-two adolescent males participated in the follow-up study and were divided into appropriate for gestational age ([AGA], n=32) and SGA (n=20). The authors were careful to avoid major selection bias. Pubertal stage, testicular size, and reproductive hormones were determined, including overnight LH and FSH profiles.

No significant differences were found in testosterone levels, inhibin B levels and LH/testosterone ratio between AGA and SGA. Neither difference was observed between both groups for testicular size and morphology (determined by ultrasonography and overnight secretory patterns of gonadotropins). Median basal LH secretory rates were two-fold higher in men born AGA but the difference did not reach statistical significance. Fetal growth during the third trimester of pregnancy did not influence any of the reproductive hormone levels nor their secretory pattern as estimated by deconvolution analysis.

This is the first study to explore the influence of the third trimester fetal growth rate on subsequent adult gonadal function. These results do not rule out the gonadal damage in relation to genital malformations as cryptorchidism and hypospadias which also occur in relation with SGA. The testicular function was not impaired in adolescent males born after compromised fetal growth hormones.

Jensen RB, Vielwerth S, Larsen T, Greisen G, Veldhuis J, Juul A. Pituitary-gonadal function in adolescent males born appropriate or small for gestational age with or without intrauterine growth restriction. J Clin Endocrinol Metab. 2007;92:1353-7.

Editor's Comment: *Most IUGR studies have focused on female reproductive function and have suggested that young women born SGA have reduced ovarian volume, decreased ovarian hormones, and increased number of anovulatory cycles.[1,2] Hyperinsulinemic insulin resistance occurring in these girls is also considered a setting for subsequent development of PCOS in adult women. The rise in FSH levels is greater during infancy in both boys and girls born SGA, whereas inhibin B levels are similar to those in infants born AGA. In adolescent males there is only limited information suggesting impaired spermatogenesis. In only one clinical study[3] of males, a significantly decreased testosterone secretion and elevated LH levels were reported, suggesting primary testicular failure in men born SGA. In 54% of those subjects, a mean testicular volume >2 SD below the control mean, with reduced inhibin B was detected; the authors considered that their data supported a link between low birth weight and reduced fertility in males born SGA. The*

presence of cryptorchidism in several cases might have played a role in the data they presented.[3] The present study provided no evidence for impaired testicular function. It may mean that whatever its cause, late fetal growth restraint is not associated with testicular dysfunction, hence there is a risk of subfertility. In a recent review[4] the limitation of information in this area has been stressed, yet many reports have dealt with connected issues such as cryptorchidism, testicular cancer, and hypospadias.

A working hypothesis would be that males with early fetal growth restrain, generally resulting in symmetric SGA, would be at greater risk. Developmental factors would play a role at this early phase of fetal growth. It would require new prospective studies in a setting similar to that reported in this paper to elucidate this hypothesis.

Raphaël Rappaport, MD

References

1. Ibanez L, Patou N, De Zegher F. J Clin Endocrinol Metab. 2000;85:2624-6.
2. Ibanez L, Patau N, Ferrer A, et al. J Clin Endocrinol Metab. 2002;87:3391-3.
3. Cicognani A, Alessandroni R, Pasini A, et al. J Pediatr. 2002;141:376–9.
4. Saenger P, Czernichow P, Hughes I, et al. Endocr Rev 2007;28:219-51.

Jeune Syndrome: Defective Intracellular Flagellar Transport

Major advancements have been made in recent years in identifying gene loci that harbor mutations responsible for human genetic disease. In many, if not most instances, the studies have begun with delineating the disease, then progressing to linkage analysis and other approaches, which eventually lead to the relevant gene locus and the mutations. In the paper discussed here, however, the authors began with a disturbance of gene function and used a bioinformatics approach to find the disease.

More specifically, Beales et al were interested in disturbances of ciliary function, the ciliopathies. Several disorders including Bardet-Biedl, oral-facial-digital type 2, Joubert, Senior-Löken, and Meckel-Grüber syndromes have recently been assigned to this group. The authors questioned if a set of minimum clinical criteria could be used to predict additional ciliopathies. After compiling a list of overlapping features, they queried the London Dysmorphology Database, which yielded a list of 10 features that would potentially predict a ciliopathy. The features included retinitis pigmentosa, polydactyly, renal cystic disease, and situs inversus. When these were ranked and used to query the database again, 25 conditions were identified as possible ciliopathies, among which was Jeune syndrome, often referred to as asphyxiating thoracic dysplasia ([ATD], OMIM 208500).

ATD is an autosomal recessive bone dysplasia characterized by limb shortening, constricted thoracic cage and respiratory insufficiency in infancy. Other features often include polydactyly, cystic renal disease, and retinal degeneration. ATD is known to be genetically heterogeneous with one locus at chromosome 15q13. The authors ascertained and studied 3 families with linkage to a second locus at chromosome 3q24-3q26. One of the candidate genes in this region encodes WDR56, a protein that has been identified originally as expressed in C. elegans ciliary cells. Mutation analysis revealed a single amino acid deletion and 2 missense mutations in the 3 ATD families. Additional mutations were not detected in other patients with ATD and none of the patients with ATD who had WDR56 mutations exhibited extraskeletal manifestations of ATD.

WDR56 is conserved across species and has been renamed IFT80. It encodes a component of intraflagellar transport complex B and is essential for development and maintenance of motile and sensory cilia. To investigate its function further, the authors "knocked down" its expression in developing zebrafish. The treatment disturbed tail, kidney, and heart development and was consistent with a disturbance of hedgehog signaling in the developing fish. The authors suggested that their bioinformatics approach may lead to identification of other ciliopathies.

Beales PL, Bland E, Tobin JL, et al. IFT80, which encodes a conserved intraflagellar transport protein, is mutated in Jeune asphyxiating thoracic dystrophy. Nat Genet. 2007;39:727-9.

Editor's Comment: *Clinicians value the London Dysmorphology Database for its diagnostic utility. This paper demonstrates another use that could be applied to other clinical phenotypes.*

William A. Horton, MD

Natural History of Noonan Syndrome

One-hundred and fifty-one subjects with Noonan syndrome from 123 families were recruited into the Noonan Syndrome Research Group at St George's University of London Hospital between 1989 and 1991. Between 2001 and 2003 all families were invited to participate in a follow-up assessment which included clinical examination, echocardiography, three-dimensional digital facial photography and analysis of the *PTPN11* gene. Of the 151 patients, 34 dropped out of the study and 10 (6.6%) died. The final study cohort comprised 112 individuals (57 males) from 92 families. Seventy of these were fully assessed and 32 partially assessed. The mean age at assessment was 25.3 years and the mean interval for follow-up was 12 years.

PTPN analysis was done in 79 individuals; mutations were found in 35%. The mutations were more likely to be found in familial cases (50%).

Height SDS at entry into the study was –2.184 and at follow-up was –1.755. Twelve individuals received growth hormone (GH) treatment. There were no statistical differences in the height SDS between those who received and those who had not received GH therapy. Those with *PTPN* mutations had similar mean height SDS. Final adult height was 167.4 cm (males) and 152.7 cm (females). When the individuals who received GH treatment were excluded, the mean final height increased to 169.8 cm (males) and 153.3 cm (females) as those who received GH were shorter. The final height in those with the *PTPN11* mutation was about 4 cm less than the others. Pulmonary stenosis was present in 65% and more prevalent in those with the mutation. No intervention was required in 58% of subjects. Hypertrophic cardiomyopathy was present in 19%; 9 of these subjects also had pulmonary stenosis. Five individuals died from complications related to hypertrophic cardiomyopathy and one person had a cardiac transplant. Feeding difficulties at ascertainment were common; some were associated with developmental speech delay. Approximately 73% of these individuals attended mainstream schools while 27% attended schools for children with learning disabilities (the mutation was equally distributed between the 2 groups); 16% had achieved higher education (this compares with 25% of the UK population). Sixty percent were full-time employed, while 26% were registered as disabled. Orthodontic work had been performed on 51%. Six percent of the individuals had required hormone injections to induce puberty. Puberty was somewhat delayed, starting at 14.5 years (males) and 14 years (females). Of those individuals who attempted to have children, 67% experienced no problems. Easy bruising or bleeding was seen in about 79%, but not associated with any known coagulopathy; prevalence of the *PTPN* mutation was higher in those with a history of easy bruising. Refractive errors were seen in 71% of the individuals. Lymphedema affected the lower limbs of 2, and scoliosis was present in 13%. Approximately 13% had recurrent seizures; *PTPN11* mutations were identified in 2 of those. All subjects, with the exception of 4, had normal hearing at follow-up.

The authors stated that their longitudinal follow-up was one of the largest databases on well-characterized Noonan syndrome. However, they have some potential bias in follow-up data because of the individuals who had dropped out. They also pointed out that they could not correlate the *PTPN* mutations with short stature as others have demonstrated. They found that about 10% of the subjects with mutations had hypertrophic cardiomyopathy.

Shaw AC, Kalidas K, Crosby AH, Jeffery S, Patton MA. The natural history of Noonan syndrome: a long-term follow-up study. Arch Dis Child. 2007:92:128-32.

Editor's Comment: *Shaw and colleagues are to be congratulated in following a large group of individuals with Noonan syndrome from childhood through adulthood and final height. Their data do not confirm the data of others with regard to mutations and short stature. Studies from France, Brazil, and Germany have demonstrated different findings with regard to Noonan syndrome. Limal et al[1] showed that individuals with the PTPN11 mutation have poor growth and do not respond to GH administration as well as those without the mutation. Ferreira et al[2] have also shown reduced GH response to long-term GH treatment. Finally, Binder et al[3] have shown that those with SHP-2 mutation have mild GH resistance and also poor GH response. Thus it is not surprising that the individuals treated with GH in the current longitudinal study, although shorter, also ended up shorter than those who had not been treated. Other studies will be needed in order to determine whether higher doses of GH or insulin like growth-factor (IGF)-I treatment[4] may enhance final height of those children with Noonan syndrome who have the PTPN11 mutation.*

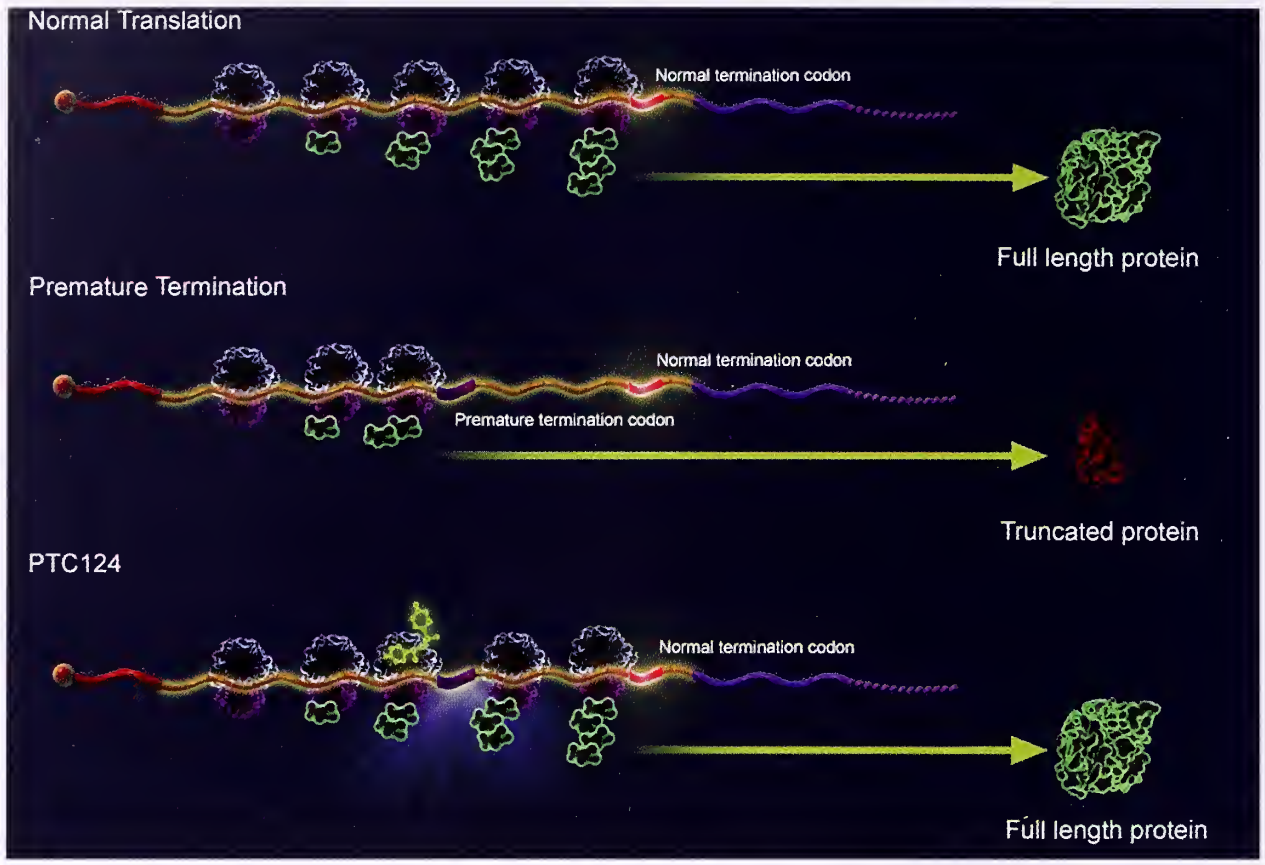
William L. Clarke, MD

References

1. Limal JM, Parfait B, Cabrol S, et al. J Clin Endocrinol Metab. 2006;91:300-6.
2. Ferreira LV, Souza SA, Arnhold IJ, et al. J Clin Endocrinol Metab. 2005;90:5156-60.
3. Binder G, Neuer K, Ranke MB, et al. J Clin Endocrinol Metab. 2005;90:5377-81.
4. Kim RJ, Grimberg A. Growth Genet Horm. 2007;23:1-7.

Nonsense Mutations in Genetic Disease—A Novel Treatment

Nonsense mutations are a common cause of human genetic disease. They give rise to in-frame premature translation termination or stop codons within the coding regions of genes and lead to truncated protein translation products that are typically nonfunctional and also promote mRNA destruction by so-called nonsense-mediated mRNA decay (NMD). The idea of developing pharmacologic means to induce a cell's translation machinery to readthrough premature termination codons (PTCs) has been around for some time, and there is evidence that the antibiotic gentamicin prompts ribosomes to readthrough PTCs to generate full-length proteins (Figure). In fact, gentamicin has received attention in this context and preliminary trials have been carried out in patients with Duchenne muscular dystrophy (DMD) and cystic fibrosis due to mutations that introduce PTCs. However, the high doses that are required, potential for renal and otic toxicity and



Translation of an mRNA into protein: comparison of normal translation, premature translation termination, and treatment with PTC124 restoring synthesis of full-length protein. Reprinted with permission from PTC Therapeutics.

need for intravenous or intramuscular administration of gentamicin have limited its potential usefulness for treatment of human diseases. A new compound has now been identified that appears to suppress PTCs with fewer potential problems.

Welch et al utilized high-throughput screening of ~800,000 compounds to identify small molecules that would suppress PTCs. One compound designated PTC124 promoted dose-dependent readthrough of PTCs, including human and mouse nonsense alleles of the dystrophin gene. Compared to gentamicin, PTC124 was effective at much lower doses and it could be delivered orally. After documenting an increase in dystrophin protein levels in primary muscle cell cultures, they then treated *mdx* mice, a mouse model of DMD due to a mutation-induced PTC in the dystrophin gene.

PTC124 treatment led to partial rescue of the muscle disturbance in the *mdx* mice. The most notable functional result was protection against susceptibility to contraction-induced injury. This injury, which is a typical feature of the *mdx* mouse and most likely occurs in DMD patients, involves repeated cycles of degeneration—regeneration, inflammation, and necrosis that eventually leads to muscle destruction. Susceptibility to this injury for mice treated with PTC124 was no different than for wild-type mice.

Mdx mice treated with PTC124 for 8 weeks showed significant decreases in serum creatinine kinase levels compared to untreated controls. Their muscle tissues displayed increased levels of dystrophin protein as well as γ-sarcoglycan consistent with production and stabilization of the dystrophin-associated membrane complex that is missing in the absence of dystrophin. Drug treatment also led to a partial restoration of dystrophin to the membrane of skeletal muscles, although the relative amount appeared to be less than in wild type mice.

The authors concluded that PTC124 is a more potent nonsense mutation suppressing agent than gentamicin. They attribute its effect to directly suppressing premature termination during translation rather than to interference with NMD. Importantly, they also provided evidence that it does not affect the function of normal termination codons. The authors suggested that through increasing the efficiency of translation, PTC124 may benefit patients with genetic diseases due to nonsense mutations. An accompanying news and views comment indicated that Phase II clinical trials are underway for PTC124 in DMD and cystic fibrosis.

Welch EM, Barton ER, Zhuo J, et al. PTC124 targets genetic disorders caused by nonsense mutations. Nature. 2007;447:87-93.

Schmitz A, Famulok M. Chemical biology: ignore the nonsense. Nature. 2007;447:42-3.

Editor's Comment: *The research described in this paper could have significant impact on the treatment of a subset of genetic disease. It reflects a marriage between so called chemical biology, which seeks to identify small molecules that produce desired therapeutic effects on disease processes, and continued efforts to understand the molecular consequences of mutations. It underscores an importance of DNA diagnoses.*

The paper raises the concern that suppressing PTCs would lead to synthesis of mutant proteins. In many instances such as enzymopathies and disorders in which structural proteins serve as platforms for or link together cellular machinery, such as in DMD, having more protein even if it harbors a mutation, would seem beneficial. However, there may also be instances where having no protein is better for a cell or a tissue than having a mutant protein that adversely affects other normal molecules.

William A. Horton, MD

Premature Menopause in Survivors of Childhood Cancer

Female childhood cancer survivors (CCS) who retain ovarian function after completing cancer treatment are at increased risk of developing premature menopause, defined as cessation of menses before age 40 years. However, the data regarding premature menopause in female CCS are scanty, although particular attention should be also paid to other endocrine alterations, and neurocognitive and neurobehavioral problems.

Sklar and colleagues assessed the incidence of and risk factors for premature menopause in 2819 CCS females older than 18 years of age who continued to menstruate for at least 5 years after their cancer diagnosis. The group was composed of control participants in the multicenter Childhood Cancer Survivor Study (CCSS), including 1065 female siblings of participants in the CCSS. Female CCS patients who received more than 30 Gy of radiation to the brain and/or had a primary tumor in the region of the hypothalamus-pituitary gland (known to cause hypogonadotropic hypogonadism) were excluded. Of 2819 subjects, 1025 had leukemia, 404 Hodgkin's lymphomas, 324 bone tumors, 297 kidney tumors, 271 sarcomas, 207 neuroblastomas, 154 non-Hodgkin's lymphomas, and 137 brain tumors. The comparison group was 1065 female siblings of participants in the CCSS. A total of 126 CCS and 32 control siblings developed nonsurgical premature menopause. The cumulative incidence of nonsurgical premature menopause was higher for CCS than control siblings (8% vs 0.8%; RR=13.21, 95% CI=3.26 to 53.51; $P<0.001$). A multiple Poisson regression model showed that risk factors for nonsurgical premature menopause included attained age, exposure to increasing dose of radiation to the ovaries, increasing alkylating agent score (based on number of alkylating agents and cumulative dose), and a diagnosis of Hodgkin's lymphoma. For female CCS subjects who were treated with alkylating agents plus abdominopelvic radiation, the cumulative incidence of nonsurgical menopause approached 30%. The results of this study will facilitate counseling current female CCS about their future risk of premature menopause and aid in designing new regimens that seek to diminish late ovarian toxicity.

Sklar CA, Mertens AC, Mitby P, et al. Premature menopause in survivors of childhood cancer: a report from the childhood cancer survivor study. J Natl Cancer Inst. 2006;98:890-6.

Editor's Comment: *This is a very interesting observational study which provides important information for physicians who care for female CCS patients. Because survival rates of cancer patients have improved markedly in recent years, the long-term complications and late effects, such as endocrine impairments and neuropsychological problems, have become increasingly important even after many years following the conclusion of treatment. The interest in the late effects of ovarian function, especially of acute ovarian failure, premature menopause and fertility, has increased over time.*

Acute ovarian failure (AOF), defined as never menstruating or premature menopause within 5 years of diagnosis of childhood cancer, is known to develop in female CCS. Chemaitilly et al[1] studied AOF in 3390 eligible female CCS in the CCSS. In this group, 215 patients (6.3%) developed AOF. Survivors who received cranial irradiation at doses of more than 30 Gy, those with hypothalamic/pituitary tumors, and survivors who underwent bilateral oophorectomy were excluded. Survivors with AOF were older at diagnosis and more likely to have been diagnosed with Hodgkin's lymphoma or to have received abdominal or pelvic radiotherapy than survivors without AOF. Among survivors with AOF, 116 (54%) had received at least 10 Gy ovarian irradiation. In a multivariable logistic regression model, increasing doses of ovarian irradiation, exposure to procarbazine, and exposure to cyclophosphamido at ages 13 to 20 years were independent risk factors for AOF.

Concerning premature menopause in CCS, Sklar and colleagues studied a total of 2819 subjects. Median age at cancer diagnosis was 7 years (range 0 to 20), and median age at study was 29 years (range 18 to 50), 69% of survivors had reached age 25 years, 47% reached age 30 years, 26% attained age 35 years, 10% were age 40 years, and 8% were older than 40 years of age. The results of their study indicated that the risk of developing nonsurgical premature menopause was 13-fold higher than that of siblings, with cumulative incidence of 8% by 40 years of age. The risk factors for nonsurgical premature menopause are: attained age, diagnosis of Hodgkin's lymphoma, and exposure to increasing doses of both alkylating agents and radiation to the ovaries.

Premature menopause and AOF leads to the early and often unexpected loss of reproduction potential as well as the cessation of ovarian sex hormone production. Thus, survivors who experience AOF or premature menopause are at increased risk of developing a variety of adverse health outcomes, including osteoporosis, cardiovascular disease, and psychosexual dysfunction.

Their results have confirmed that treatment of female childhood cancer is associated with a considerable risk of developing AOF and premature menopause. Therefore, it is necessary to inform young adult female CCS patients who are still menstruating, about the risk factors of premature menopause (ie, attained age, Hodgkin's lymphoma, chemotherapy with alkylating agents, and radiation to the ovaries) to facilitate family planning and timing of future pregnancies. It is also necessary for physicians as well as patients and family members to know that premature menopause can occur even after several years following childhood cancer treatment.

Yoshikazu Nishi, MD

Reference

1. Chemaitilly W, Mertens AC, Mitby P, et al. J Clin Endocrinol Metab. 2006;91:1723–8.

SHOX Haploinsufficiency—Clinical Indicators

Mutations in the short stature homeobox-containing gene (*SHOX*) are one of the more frequent genetic causes of growth retardation in individuals with short stature. *SHOX* is therefore an important mediator of linear growth, presenting with marked disorganization of chondrocyte proliferation. There is also a dose dependent association between the number of copies of this gene and height. In addition, *SHOX* deficiency includes a continuum of clinical conditions spanning from the severe Langer syndrome with no functional copy of the *SHOX* gene to the Leri-Weill syndrome (LWS) and isolated idiopathic short stature (ISS) without dysmorphic features and haploinsufficiency of the *SHOX* gene. The aim of this study was to determine the phenotypical spectrum of *SHOX* deficiency in a large cohort of children with short stature and to propose a scoring system to select patients who qualify for diagnostic SHOX molecular testing.

A cohort of 1608 unrelated prepubertal children with short stature was studied. Investigators were requested to report the presence or absence of a number of dysmorphic signs often observed in LWS. In addition, for sake of comparison, a group of Turner syndrome patients (n=33) was included as this condition also presents one absent *SHOX* allele. *SHOX* defects were identified in 68 short stature participants (4.2%) and in 34 ISS patients (2.2%). LWS was reported in 55 participants (3.4%).

For LWS patients, the presence of a *SHOX* defect did not induce a greater frequency of dysmorphic signs. For ISS children, a number of physical signs (shortening or bowing of the forearm, Madelung deformity [dinner fork-like deformity of the wrist], dislocation of the ulna of the elbow, high-arched palate, bowing of the tibia and appearance of muscular hypertrophy) were clinical indicators for *SHOX* deficiency. In addition, ISS patients presented a significantly greater BMI SDS than those without the molecular defect. They also they had disproportion and appearance of muscular hypertrophy; ISS with *SHOX* deficiency had a significantly greater sitting height and forearm, upper arm and upper leg circumference adjusted for standing height.

The variability of the phenotype makes it difficult to decide molecular testing. Therefore various logistic regression models were developed. The diagnosis of LWS or the presence of Madelung deformity resulted in very high odds ratios. Therefore the study focused on non-syndromic ISS, and these strong indicators were excluded from the models. A scoring system was presented (Table) using rounded odds ratios as weighted score points to help identify patients who qualify for *SHOX* gene testing based on clinical criteria. These criteria included 3 anthropomorphic variables (arm span/height, sitting height/height, and BMI) and 5 dysmorphic signs (cubitus valgus, short forearm, bowing of forearm, appearance of muscular hypertrophy, and dislocation of the ulna at the elbow). Testing was recommended for patients having a score greater than 4 or 7 out of a total possible score of 24, a range that allows a better sensitivity.

This study provided clinical guidelines for testing of the *SHOX* gene. The most reliable clinical indicators of *SHOX* deficiency were related to disproportionate growth. The clinical findings were present in a significant number of ISS phenotype patients as well as in those with LWS. These diagnoses represent a continuum rather than discrete entities. However, there was no clear correlation between the specific *SHOX* gene defect and the clinical features.

Rappold G, Blum WF, Shavrikova EP, et al. Genotypes and phenotypes in children with short stature: clinical indicators of SHOX haploinsufficiency. J Med Genet. 2007;44:306-13.

Editor's Comment: *SHOX haploinsufficiency is an important genetic cause of short stature associated with well recognized dysmorphic signs. Several studies have already reported on this clinical phenotype. The present report provided new data prospectively established from a large group of patients with short stature. This analysis should help to identify the large continuum of phenotypes related to SHOX haploinsufficiency among the various types of short stature. The gene-phenotype relationship is not a simple one, as the authors found no difference in the degree of short stature between the children with or without an identifiable SHOX defect (according to the molecular methods applied in this protocol). Furthermore, among short stature patients with a clinical diagnosis of LWS the frequency of dysmorphic signs was not different between children with a SHOX defect and those without an intragenic mutation of the SHOX gene. Other genetic defects, not investigated in this study, might have been missed: intragenic mutations located in regulatory regions of*

Scoring system for identifying patients that qualify for short-stature homeobox containing gene *(SHOX)* testing based on clinical criteria

Score item	Criterion	Score points
Arm span/height ratio	<96.5%	2
Sitting height/height ratio	>55.5%	2
Body–mass index	>50th percentile	4
Cubitus valgus	Yes	2
Short forearm	Yes	3
Bowing of forearm	Yes	3
Appearance of muscular hypertrophy	Yes	3
Dislocation of ulna (at elbow)	Yes	5
Total	24	

the gene, or defects at a different, but related, genetic locus. It is of interest that, for an unknown reason, BMI is increased in those patients with muscular hypertrophy. This finding is in contrast with the frequency of low to low-normal BMI values in children with ISS. A recent report[1] showed that similar stimulation of growth was obtained in ISS with SHOX insufficiency and in a group of girls with Turner syndrome who received growth hormone treatment of 50 µg/kg/day. This study also showed the importance of carefully analyzed familial histories using clinical scoring and radiographic examination of the forearm and hand.

Raphaël Rappaport, MD

Reference

1. Blum WF, Crowe BJ, Quigley CA, et al. J Clin Endocrinol Metab. 2007;92:219-28.

Stem Cells: A New Kind of Breakthrough

Stem cells have received much attention in recent years because of their potential to regenerate damaged and diseased tissues. Two types of stem cells have been distinguished historically—embryonic stem cells (ESC) and adult stem cells. Because of the potential to differentiate into any cell type, pluripotentiality, the former have more potential in regenerative medicine than the latter. In fact, this principle is illustrated well in knock-in and knock-out mice in which new mouse strains are generated from ESC into which mutations have been introduced. However, serious ethical issues are raised in human ESC research since until now, they could only be obtained from human embryos. Moreover, if this technology is to be applied to adult disease, sometimes referred to as custom transplantation therapy, means must be developed to produce cells equivalent to ESC from the patient needing treatment, which has raised controversial issues of human cloning with its own set of ethical concerns. Attempts to convert easily accessible cells such as fibroblasts to ESC-like cells have been unsuccessful until now. But 3 papers have recently been published which signal a major breakthrough in the field.

The recent story starts with the realization that converting somatic cells to ESC-like cells requires nuclear or epigenetic reprogramming of the cells, ie, resetting of DNA methylation, histone modification and chromatin structure, to that of ESC. Last year a Japanese group headed by Yamanaka[1] generated ESC-like cells from mouse embryonic fibroblasts by expressing 4 transcription factors (Oct3/4, Sox2, c-myc and Klf 4) and subsequently selecting cells that expressed another transcription factor, Fbx15. The concept was that the 4 transcription factors would trigger expression of genes highlighted by Fbx15 that induced the pluripotent state, and the cells were termed induced pluropotent stem cells or iPS cells. While these cells exhibited many stem cell properties, they were not fully reprogrammed epigenetically and did not produce chimeras when injected into mouse embryos. Chimeras are mice containing cells from the recipient mouse embryo and cells from a donor source—iPS cells in this case.

Technical modifications have now led to a second generation of mouse iPS cells with properties that more closely approximate those of ESC, including the ability to produce chimeras in the next generation of mice. The work was reported by Okita et al, Wernig et al and Maherali et al. Although each group differed in certain methodologic details, their common protocol started as before, but utilized different transcription factors—Nanog and Oct4—to identify and isolate iPS cells.

The second generation iPS cells possessed an epigenetic signature remarkably similar to ESC; they differentiated into cells of different lineages and germ layers and all 3 groups were able to establish chimeras and in 2 cases germ-line transmission in the next generation, the most stringent evidence of developmental potency.

Two concerns were raised relative to the potential use of this technology in humans. One is the development of tumors in nearly 15% of mice derived from iPS cells. This risk was attributed to expression of c-myc, which is a known oncogene and possibly reactivation of its expression at a later time. The second and related issue is the use of retroviral vectors to introduce the transcription factors that trigger nuclear reprogramming. They may predispose to oncogenesis through insertional mutagenesis as well. However, one of the conclusions from the studies is that the triggering mechanism may require only transient expression of the transcription factors, ie, once initiated, reprogramming may drive itself. If so, then transient expression, perhaps by using adenoviral vectors with less risk of problems, may suffice. All groups acknowledge that application of this technology to humans is still some time away.

Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature. 2007 [epub ahead of print].

Wernig M, Meissner A, Foreman R, et al. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature. 2007 [epub ahead of print].

Maherali N, Sridharan R, Xie W, et al. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell. 2007;1:55-70.

Editor's Comment: *These reports are very encouraging and give regenerative medicine a major boost.[2] But as stated repeatedly, more research will be needed to translate this breakthrough to the clinic. Nevertheless, the findings and especially that the three groups are able to confirm each other's results are very exciting.*

William A. Horton, MD

References

1. Takahshi K, Yamanaka S. Cell. 2006;126:663-76.
2. Cyranoski D. Nature. 2007;447:618-9.

Transition to Adulthood of Growth Hormone Deficient Children

This "Approach to the Patient" review presented a discussion on continuing care of growth hormone deficient (GHD) patients who have attained final adult height and have low bone mineral density, abnormal lipids, and impaired cardiac function. Radovick and DiVall delineated when to retest, how to test, when to treat, and how to treat. In their conclusion they recommended the establishment of specialized clinics "to improve compliance and follow-up during the transition to adult services."

Radovick S, DiVall S. Approach to the growth hormone deficient child during transition to adulthood. J Clin Endocrinol Metab. 2007;92:1195-200.

Editor's Comment: *This is an extremely detailed article addressing the important issues of transitional care of the GHD deficient patient following completion of linear growth. I cannot fault the careful objective analysis of the published literature and the recommendations are sensible and logically argued. However, this article emphasizes the very wide transatlantic divide in the approach to this subject.*

The authors described the clinical situation of the care of a GHD 17-year-old patient as being "relatively new to pediatric endocrinologists." The entity of the adult GHD syndrome and evidence of beneficial GH replacement therapy in adult hypopituitary patients was established in the late 1980s. That is nearly 20 years ago. Active transitional care programs have been operating in Europe, admittedly more so in northern rather than southern parts of the continent, for at least 10 years.

The challenge is to get pediatric and adult endocrinologists to work together to improve care of the patient with hypopituitarism who has completed growth. With this aim, a consensus meeting, organized jointly by ESPE, LWPES, and the GRS (Manchester, UK, December 2003) and its consensus statement[1] was published in 2005. This statement, authored by an equal number of pediatric and adult endocrinologists discussed all the questions raised in the present review. However the Clayton et al paper is not even referenced!

The Radovick and DiVall review is written very much for the practicing pediatric endocrinologist. This again points to a difference in approach to the care of the young adult with GHD. The consensus view, as previously published,[1] is that care should be transferred, ie, the patient should be transitioned to the adult endocrine service. In this way, GH retesting using an insulin tolerance test (ITT), which is recognized to be the best test of adult GHD, can be performed in a safe adult environment and the decision whether to continue GH therapy in a young adult, can be taken, logically, by an adult endocrinologist.

I make a plea for transatlantic co-operation. We have a great deal to learn from each other.

Martin O. Savage, MD

Reference

1. Clayton PE, Cuneo RC, Juul A, et al. Eur J Endocrinol. 2005;152:165-70.

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